

Historic, Archive Document

Do not assume content reflects current
scientific knowledge, policies, or practices.

A280.39
R313
cop. 2

As ref list 2 0477

-

**REPORT OF THE
NORTHERN MARKETING AND NUTRITION RESEARCH DIVISION
July 1, 1971**

U. S. DEPT. OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY
RECEIVED

JUN 15 1972

PROCUREMENT SECTION
CURRENT SERIAL RECORDS

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

CONTENTS

	<u>Page</u>
Introduction	1
Corn Utilization	20
Wheat Utilization (Northern Region)	62
Grain Sorghum Utilization	68
Soybean Utilization	71
Flax Utilization	86
Crambe Utilization	91
New Crops Utilization	96
Forage Utilization (Northern Region)	103

Trade names or company names are used in this publication solely for the purpose of providing specific information. Mention of them does not constitute their guarantee or warranty by the U.S. Department of Agriculture or an endorsement by the Department over others not mentioned.

Issued January 1972

PROGRESS REPORT
OF THE
NORTHERN MARKETING AND NUTRITION RESEARCH DIVISION
JULY 1, 1971

INTRODUCTION

The Northern Marketing and Nutrition Research Division, located at Peoria, Illinois, is one of five research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Marketing and Nutrition Research Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, the Southeastern at Athens, Georgia, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for the Department's utilization research on corn, grain sorghum, soybeans, flax, crambe, and new crops. Its research on wheat emphasizes industrial utilization and milling technology, and that on forages is limited to a search for the cause of toxicity occasionally displayed by tall fescue grass. Responsibility for research on food and feed utilization of wheat and for the Department's primary utilization research program on forages is assigned to the Western Division.

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation.

The summaries of progress of research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to those having a special interest in the development of public agricultural research programs.

This report also includes a list of publications and patents issued July 1, 1970 through June 30, 1971.

Following are some of the recent utilization research accomplishments of the Northern Division.

VERSATILE FOOD BLEND FOR FOREIGN PROGRAMS

Department scientists have developed processing technology for making an instant cereal food blend which, when prepared by users, provides either a highly nutritious food drink or a gruel or porridge with high caloric density. The blend, which includes corn, soy flour, nonfat dry milk, minerals, and vitamins, maintains the composition of regular CSM. The key step in the new process is treating the cereal component of the blend in extruders in such a manner that the starchy portion of the cereal is fully cooked and has low viscosity and high solubility properties. Other cereals, such as sorghum, rice or millet, or a tuberous starch source, such as cassava, can be used in place of corn. Other ingredients like sugar, salt, flavorings, or spices can be added to the basic blend to suit the taste of the user.

Wide usage of the food blend can be expected, for it may be used as a supplement to breast feeding; as a weaning food; or as a regular diet for all young children after weaning. In addition, the food blend can fill a need for supplying older children and adults with a nutritious beverage or porridge base.

The Department is purchasing multi-ton quantities of a gruel version of this versatile corn-soya-milk food blend for distribution in its foreign emergency food programs. The product also has good potential for domestic usage.

Versatile High Caloric Food Blends for Foreign Feeding Programs



INSTANT
CORN SOYA MILK (CSM)



FURNISHED BY THE PEOPLE
OF THE
UNITED STATES OF AMERICA
NOT TO BE SOLD OR EXCHANGED

WEIGHT:

NET 50 POUNDS 22.68 KILOS

CONTRACT NO. MP FF 20767
IRT SACK FABRICATED 8-71

USE NO HOOKS

UNICEF
TA-1624

- Provides a balanced food for entire family
- A highly nutritious, fully cooked weaning food for the nursing baby
- An enriched beverage base, which can be flavored to taste, for children, adolescents and adults

- A high caloric density blend, that can be prepared in the traditional manner of the country, for all family members

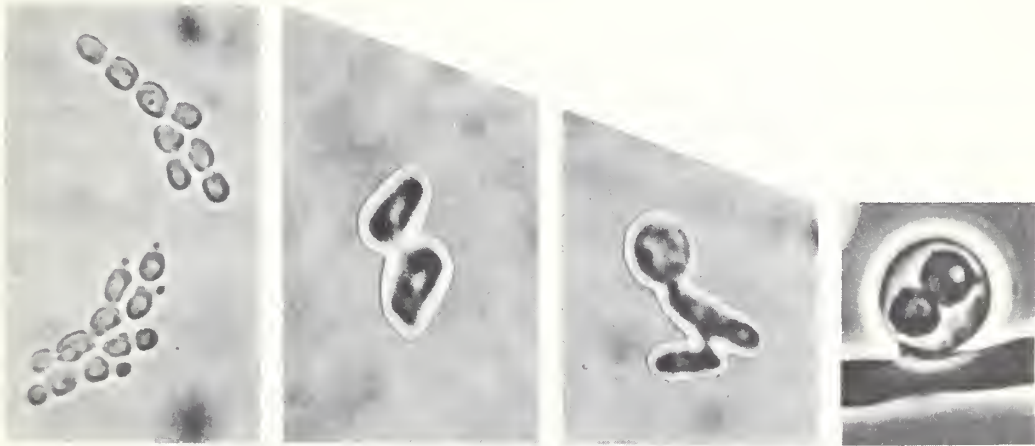


USDA helps feed the hungry of the world
through its foreign donations program

BREEDING YEASTS FOR FOOD AND FEED PROTEIN

Department scientists have discovered mating strains of a yeast commonly grown as a source of protein for food and feed. Sexual reproduction was previously unknown for this yeast. Now it should be possible to breed (hybridize) for strains that will produce increased yield of higher quality food and feed protein. Protein deficiency in many countries throughout the world has greatly stimulated interest in using microorganisms as a source of protein for food and feed. Various types of processing wastes, as well as petroleum, are some of the more promising materials on which these microorganisms are grown. Besides producing protein, growth of these microorganisms can, therefore, contribute to reducing pollution by converting wastes to more valuable products. Researchers throughout the world have requested cultures of the new mating strains.

Mating Reaction Discovered in the Yeast *Candida lipolytica*



- ▶ Breeding of yeast strains that have larger amounts of better quality protein for supplementing foods and feeds.
- ▶ Increased utilization of waste materials.

RAPID METHOD FOR DETECTING AFLATOXIN IN CORN

A simple, rapid method for detecting carcinogenic aflatoxin in corn has been discovered by Department scientists. Damaged corn kernels, corn particles, and particles of foreign matter in corn, when illuminated with ultraviolet light, have been found to glow with a characteristic greenish-gold fluorescence if they contain significant levels of aflatoxin. No sample of whole, undamaged corn has yet been found to contain aflatoxin. Surveys have revealed only a low incidence and level of aflatoxin mostly in poor grades of commercial corn. However, because aflatoxin is a powerful carcinogen, it is important to identify rapidly any suspect samples, which then can be examined in greater detail. Such identification can now be easily and quickly made even by personnel with minimal training. The availability of this new rapid method for detecting aflatoxin is expected to speed up the movement of corn in commerce and to provide more positive assurance that corn used for food and feed is free from contamination with aflatoxin.

Rapid Method for Detecting Aflatoxin in Corn



**Fluorescence of Corn Kernels Reveals
Probable Presence of Aflatoxin**

EASY-QUICK-CONVENIENT ROUTINE TEST

QUALITY OF MILLED PRODUCTS FROM BLIGHTED CORN REMAINS GENERALLY GOOD

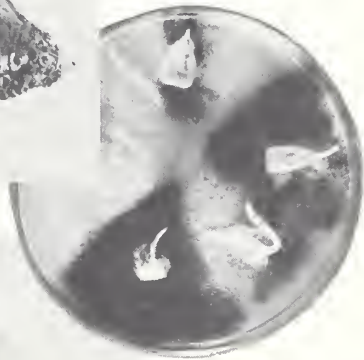
Department scientists have shown that milled products from corn affected by southern corn leaf blight retain good quality. Although blighted corn grades lower in quality than uninfected corn, both wet-milled products (starch and oil) and dry-milled products (grits and meal) were generally indistinguishable from similar products milled from uninfected corn. Crude oil from blighted corn contained more than the normal amount of free fatty acid, which, however, could be readily removed during refining. Taste panel tests revealed no flavor or odor problems. The only disadvantage observed with blighted corn was that yields of products decreased as blight damage increased. There was no evidence of a toxicity problem due to the blight itself, providing no subsequent infection with other toxin-producing molds had occurred.

Severe losses were widespread in the Corn Belt in 1970 because of infection with a new race of southern corn leaf blight, Helminthosporium maydis Race T. Other than the decreased yields of milled products, blighted corn should be as useful as unblighted corn for commercial purposes.

Utilizing Blight-Damaged Corn in Industrial Processing



Blight-damaged ear



H. maydis
growing out
from corn

H. maydis spores



Normal recoveries of starch and oil in wet milling process except from most heavily infected corn. Product quality remained good.

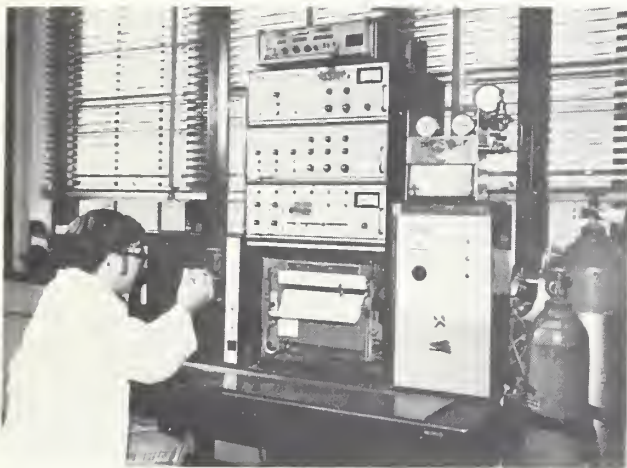
Dry milling yields decreased for more heavily infected corn.



NEW ANALYTICAL TOOL SPEEDS RESEARCH ON ABATEMENT
OF POLLUTION BY FEEDLOT WASTES

A new way to analyze feedlot wastes for carbohydrate, developed by Department scientists, promises to speed research on abatement of pollution by such wastes. Animal wastes contain 35 to 50 percent of carbohydrate in which cellulose and hemicellulose predominate. These fibrous carbohydrates contribute significantly to the chemical oxygen demand in biological disposal systems. If the wastes are refeed, these carbohydrates are poorly digested by nonruminants and only partly digested by ruminants on a high energy ration. Systems to degrade these carbohydrates are urgently needed for satisfactory disposal of the wastes. In contrast to the tedious, time-consuming, inaccurate conventional procedures to determine carbohydrate, the new method is simple, accurate, convenient, and relatively rapid. Research to devise satisfactory methods for degrading cellulose and hemicellulose and to ascertain the fate of these carbohydrates in animal waste disposal systems should proceed much more rapidly by use of the new analytical method. This method of analysis is also finding general applicability in other areas, such as the analysis of wood pulp and determining the fiber content of foods and feed.

GLC Aids Fight Against Feedlot Waste Pollution



**Carbohydrates Are
35-50% of the
Pollution Potential
in Animal Wastes**

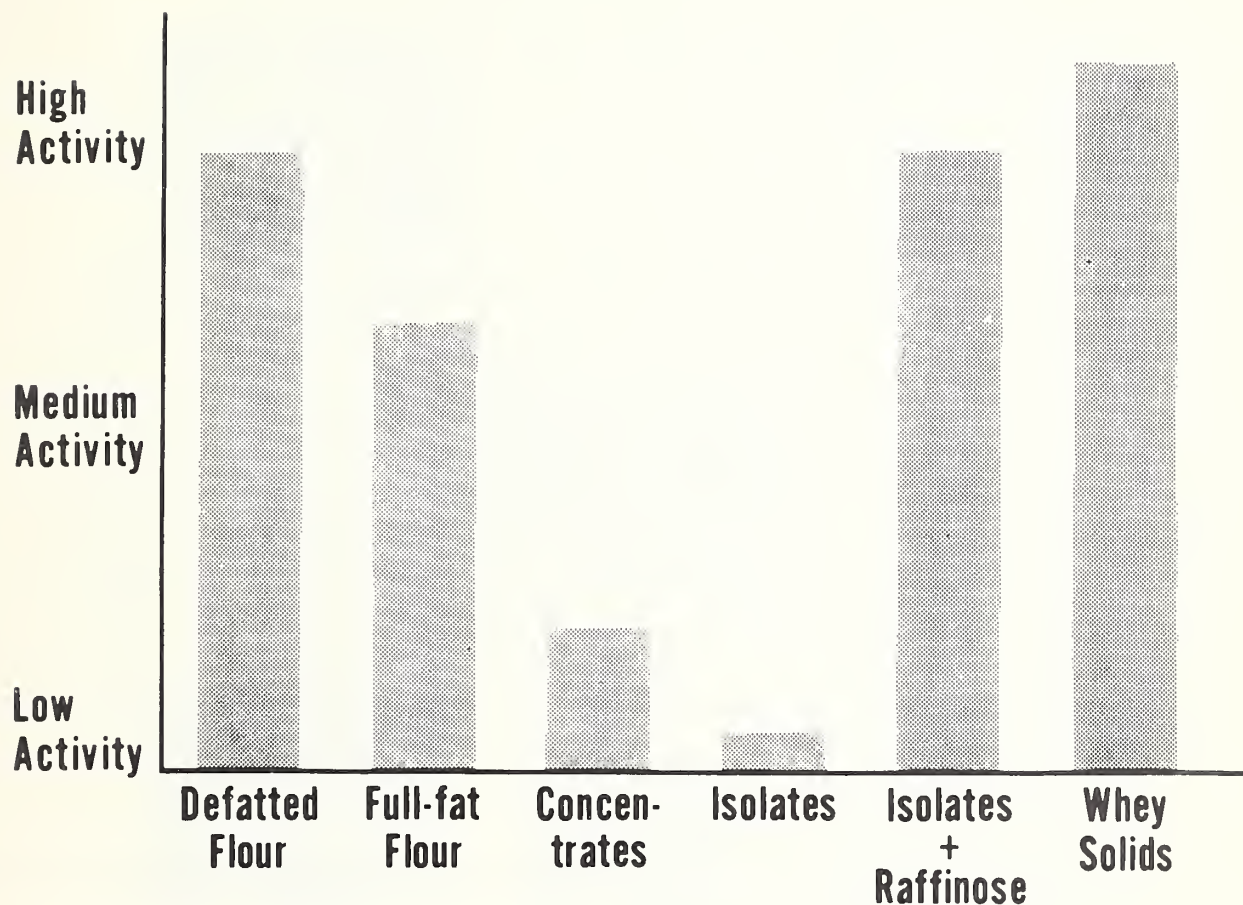
**Analytical tool helps to eliminate these
carbohydrates by guiding technological
developments in waste treatment**

IMPROVED SOYBEAN PROTEIN PRODUCTS FOR FOODS

USDA-sponsored research at the University of Illinois in cooperation with ARS scientists has revealed the cause of flatulence produced by several soybean food products.

When acute problems with flatus arose after reducing-diet beverages containing soy flour became popular in the 1960's, soy flour in these products was reduced to low levels or replaced by other protein sources. Flatus activity of full-fat and defatted soy flours has now been shown to be caused by sugars, called raffinose and stachyose, which are resistant to the enzymes produced by the human digestive tract. As a result, the sugars are not absorbed and pass into the lower intestinal tract where they are fermented by bacteria to form the primary flatus components: carbon dioxide, hydrogen, and methane. Further processing of soy flour produces protein concentrates or isolates, both commercially available, which contain less raffinose and stachyose. Soy protein concentrates, containing 70 percent protein, have one-half or less of the flatus activity of the flours. Protein isolates, which are nearly pure protein, have almost no flatus activity. Soybean whey, the byproduct of isolate manufacture, contains raffinose and stachyose and consequently has very high flatus-producing activity. Present estimates of annual food uses of soy flours, concentrates, and isolates are 121, 33, and 38 million pounds, respectively. Information now available reveals routes to possible methods of removal of flatus factors and promises to enhance the position of soy protein products as ingredients for fabricated foods and to lead to their expanded use.

Flatulence Activity of Soybean Products



Accomplished Objectives:

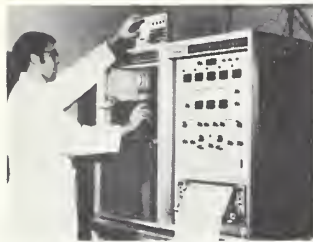
- ▶ Flatulent properties of soy products measured
- ▶ Flatus factors and mechanism of action identified
- ▶ Routes to possible methods of removal of flatus factors revealed

COMPUTER GUIDES RESEARCH OF AGRICULTURAL SCIENTISTS

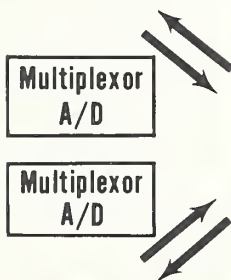
Research of scientists at the Northern Division is now guided by a computer operating in "real time." In "real time" or "on-line" operation the computer is connected to and receives data directly from operating scientific equipment. The computer almost instantaneously compares experimental results with theory or other predetermined criteria and returns the result of that comparison to the scientist via a teleprinter at the location of the experiment. The scientist thus knows at once if the range, number, and accuracy of measurements have been sufficient. If not, he can immediately make the necessary adjustments in the experimental parameters. This new approach contrasts with the conventional one, that of waiting an indefinite period of time--days or perhaps weeks--before the adequacy of an experiment could be known. At the Northern Division, equipment now connected to the computer includes gas chromatographs, mass spectrometers, and even a miniature soybean oil refinery. With the aid of "real time" operation, the scientist now benefits from greatly increased efficiency in experimenting with such practical problems as controlling a vegetable oil refinery; evaluating selective hydrogenation catalysts for soybean oil; producing edible fats high in polyunsaturation; learning the metabolic fate of isomeric fats tagged with radioactive isotopes; and identifying odors of heated or aged edible oils.

COMPUTER-GUIDED RESEARCH

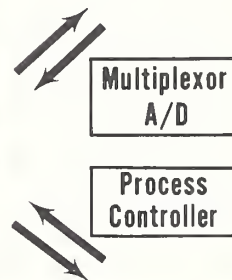
Real Time Mode



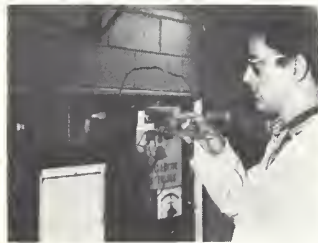
**Polyunsaturates
in Edible Fats**



**Metabolic Fate of
Isomeric Fats**



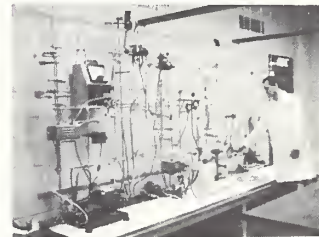
Central Laboratory Computer



**Catalyst Evaluation for
Hydrogenating Soybean Oil**



Satellite Computer



**"Mini-"
Soybean Oil Refinery**



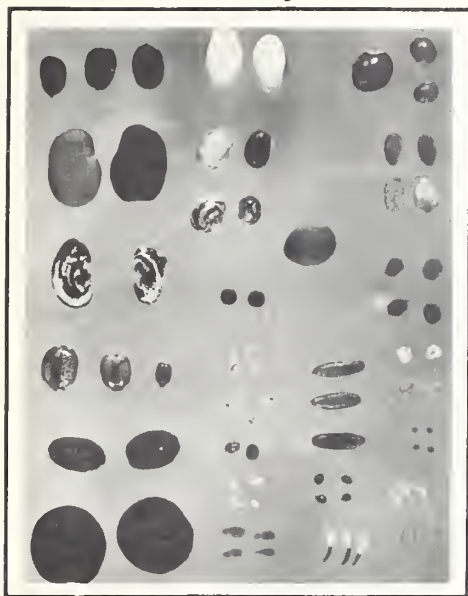
**Odor Identification by Mass
Spectrometer and Smell**

NEW SEED OILS AS FUTURE RAW MATERIALS

Interdisciplinary research involving close cooperation among Department botanists, chemists, and agronomists is uncovering prospective new agricultural products having a wide range of useful properties and possible applications. With the primary objective of developing new crops, this project is yielding discoveries of previously unknown seed oil constituents at an average rate approximating one per hundred species of uncultivated plants investigated. So far, it has resulted in over 60 additions to the repertoire of diverse natural chemical entities accessible for technological utilization. Besides the ones containing these substances identified for the first time, other new seed oils have been found to be exceptionally rich sources of already known compounds of economic interest. Fifteen of the new seed oils discovered in the project could serve in current industrial uses, and at least six are known to be receiving some degree of attention by one or more commercial firms. Conceivably more important in the long run, an extensive array of versatile raw materials potentially available from agriculture is being accumulated that will provide valuable insurance against possible future needs to supplement non-renewable chemical resources.

Interdisciplinary Research Seeks New Oilseed Crops

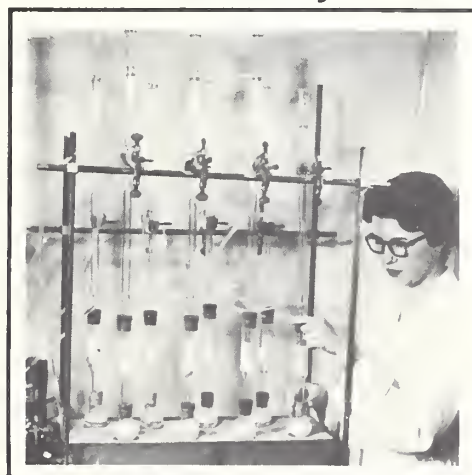
Botany



Agronomy



Chemistry



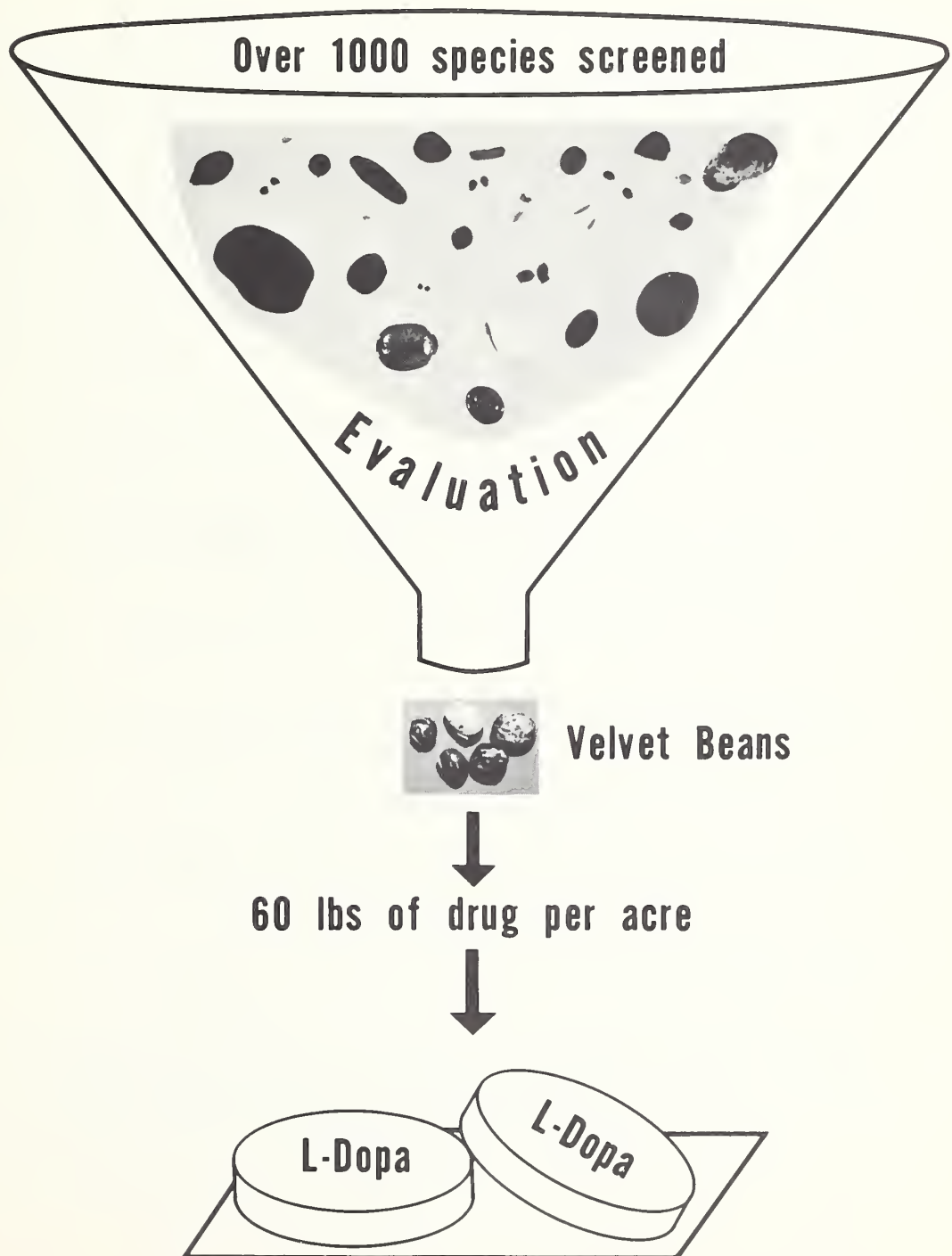
Potential New Raw Materials for Industry

L-DOPA IN SEEDS

How much and in which species? Research by Department scientists has answered these questions and developed a method for recovery of virtually all of the L-dopa from the richest sources. This new drug dramatically alleviates symptoms in most cases of Parkinson's disease. Although several companies have received or applied for FDA approval to market it, the best route for commercial production remains moot. Chemical synthesis gives a difficultly separable mixture of the medically useful L-dopa and the undesirable D-form. A fermentation procedure avoids this problem but requires a relatively expensive starting material. The Department study has provided valuable information to help interested firms assess the merits of extraction from seeds as a feasible alternative.

Of seeds from more than 1,000 species analyzed, only velvet beans and close relatives contained enough L-dopa to warrant consideration as a commercial source. Many seeds reputed to contain the substance, such as broad beans and sesame, actually had only traces of it. On the other hand, the level in velvet beans was near 4.5 percent, or more than twice as much as previously reported, and a few close relatives contained significantly more (up to 10 percent in one sample). Unfortunately, the relatives are at present not well adapted to American agriculture, but velvet beans grown in the United States have produced 1,500 pounds per acre. This yield coupled with the new Department recovery method could afford 60 pounds of the valuable drug per acre.

Seed Source of Drug for Parkinson's Disease



CORN UTILIZATION

Problems and Objectives

Corn is the major source of the 3 billion pounds of cereal starches and flours used annually in the U.S. for industrial purposes. Synthetic products from nonagricultural sources constantly threaten these agricultural outlets. A continuing effort is required to develop new and improved products with better performance capabilities in industrial products such as adhesives, chemicals, elastomers, paper, plastics, protective coatings, and thickening agents. Nearly 80% of the U.S. production of corn is used as animal feed. The size of this outlet provides direct gains to farmers and to industry through even small improvements in grain quality or processing efficiency. Food use of corn also represents an economically important market, and significant benefits can be derived from developments leading to cheaper and more healthful products.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Find new technologies for the conversion of starch by chemical or fermentative processes to products for use in the paper, rubber, chemical, and other industries.
2. Provide increased industrial outlets for new classes of high-amylose corn.
3. Develop low-cost foods from new corn varieties having higher nutritional value than ordinary dent corn.
4. Improve yields and quality of corn dry-milling fractions.
5. Develop more acceptable, varied, and nutritious foods from corn.
6. Remove mold toxins or harmful microorganisms from corn and corn products.

Work in several areas of research on corn was curtailed temporarily in order to direct effort to problems arising from outbreak of Southern Corn Leaf Blight. All research findings concerned with blighted corn are summarized under Section C, "Protecting Food Supplies From Harmful Organisms."

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Milling Artificially Dried Corn

Purpose: Increasingly, corn is being field harvested at high moisture contents. Such corn requires artificial drying which lowers its quality for dry-milling purposes. A suitable dry-milling process is needed to improve yields, quality, and size of grits.

Progress: To determine the effect of selected variables in roller mill operation upon yields and fat contents of milled products from artificially dried corn, roll differential, roll gap, and type of roll action are being studied at four levels. Results to date indicate that a 1.5:1 differential, 0.025-in. gap, and dull-to-dull roll action would be the best overall choice for second break roll operation.

2. Conversion of Starch to Sugars and Sirups

Purpose: Corn starch is a significant source of sweeteners used by the food industry. Processing is the major factor in the cost of such sweeteners. To meet increasing demands for sweeteners of high quality, methods are needed for converting starch to sugars and sirups continuously and with fewer reversion products.

Progress: Recent commercial development of enzymatic starch hydrolyses to produce 90-95% maltose sirups has prompted further investigations into the preparation and properties of various solid forms of maltose. Simple ways were found for crystallizing aqueous 90-95% maltose sirups to produce anhydrous, nonhygroscopic, crystalline maltose. Widely differing hygroscopicities for the beta-monohydrate; anhydrous beta; anhydrous alpha, beta (70-95% alpha); and amorphous spray-dried forms were determined at 31, 52, and 84% relative humidity. Solubilities were determined for the unanomericized anomeric forms, and varying anomeric compositions were determined at equilibrium in different solvents by GLC. A commercial amylopectin containing a high percentage of maltooligosaccharides was fractionated in various ways to yield dextrose-free fractions consisting of oligosaccharides with degree of polymerization less than 10. New acetals and orthoesters of dextrose, maltose, and methyl hexopyranosides were prepared to define their ring conformations through NMR analyses, to prepare 2-O-substituted derivatives of dextrose and maltose, and to correlate sweet taste with spatial orientations of glycol groups. Amine-catalyzed isomerizations of maltose to maltulose were conducted and a pure maltulose fraction was isolated by ion-exchange chromatography.

Glucosylase ionically bound to DEAE cellulose continuously converted 30% corn starch to glucose in a stirred flow reactor. Yields were comparable to those obtained with soluble enzyme. A stable preparation of bacterial α -amylase covalently bound to porous glass beads degraded starch continuously in a column percolate. Steroid esterase bound to glass is more stable than the free enzyme. This is also true for glass-bound glucose isomerase. As previously shown for isomerase entrapped in a gel matrix, continued changing of substrate solution in a column system extends the usable life of the enzyme. Divalent cations were shown to markedly affect 3-keto-disaccharide production by resting cells of Agrobacterium tumefaciens. For example, studies with C-14-labeled disaccharide showed that Zn^{++} inhibited the rate of uptake. Washing the cells with EDTA or adding Mg^{++} reversed inhibition by Zn^{++} . The 3-ketosaccharide synthesizing enzyme itself was unaffected by all divalent cations tested except Cu^{++} . The degree of tolerance of yeast to $Cr(III)$ does not correlate with ability to produce glucose tolerance factor (GTF). A variety of genera can produce moderate amounts of GTF. Incorporation of yeast extract and phosphate in the culture medium appears to stimulate growth of Saccharomyces carlsbergensis as well as GTF production by this yeast.

In grant studies at Iowa State University, Ames, the amylase structural gene of Bacillus subtilis has been located on the chromosome near the structural gene for chorismic acid synthetase. This finding should facilitate further genetic work with the amylase of B. subtilis. A mutant of B. subtilis requiring glutamic acid for growth was obtained and used in experiments attempting to grow wall-less cells. This was unsuccessful, but protoplasts of the mutant could be converted to coccal forms having extremely thick walls that did not produce amylase but did produce protease. This is indirect evidence that the wall may play a role in amylase formation. Production of B. macerans transglycosylase has been significantly improved by the discovery that dextrans of about DP 40 can be directly transported into the cell where induction of the enzyme purportedly takes place. Starch and other high molecular-weight glycans are poor inducers because they must be broken down before they can enter the cell. Likewise, small oligosaccharides such as DP 9 and smaller, though transported into the cells, are not of themselves good inducers. This finding brings the time required for biosynthesis of this enzyme down from 4 weeks to 4 days with increased transglycosylase yields.

Computer simulation studies of α -amylase action on small oligosaccharides have been made under a grant to the University of Arkansas, Fayetteville, to test the proposed subsite model of the enzyme. The following conclusions seem justified:

(a) The subsite model accounts for both the chain length dependence of Michaelis parameters and product patterns for substrates of 1 to 12 glucose units, (b) subsite VI has an interaction energy of +3000 cal/mole which strains the substrate during catalysis, and (c) the other subsites contribute to the substrate strain and therefore enhance the catalytic efficiency of the α -amylase.

Experiments with radioactive glucose under a grant to the University of Minnesota demonstrated that, in all probability, media ingredients are taken up at the hyphal tips of mold mycelium and remain there and that cytoplasmic streaming does not result in transport of cellular constituents or nutrients. This was shown by taking germinating spores of Aspergillus awamori and pulsing them with radioactive glucose followed by return to unlabelled glucose for varying lengths of time. The results indicated that the label stayed in that portion of the mycelium that was a hyphal tip at the time of pulsing. The possibility remains that the radioactive label is due to incorporation of glucose only in cell wall constituents that have little or no turnover. However, there is ample evidence from the literature that external glucose readily reaches internal cell constituents and fluids.

Under a grant to Kansas State University, Manhattan, a new application of electrophoretic moving boundary theory was derived as a general method for separating and purifying proteins and enzymes. The method has been termed "stacking" electrophoresis and employs electrophoretic moving boundaries induced by localized ionic discontinuities. The process is conducted in a column stabilized by Sephadex or cellulose powder. Separation and resolution follow the Kohlrausch regulating function, and the resolved protein bands are adjacent to one another. Success of the separation process is dependent upon the trailing ion properties. The most useful ions in large-scale separations have been amine and carboxyl derivatives of polyoxyethylene which replace the amphoteric ions used in the usual disc electrophoresis separations. With these polyoxyethylene ions, relatively low potentials can be used and forced cooling often avoided. The largest system attempted was the separation of 1 liter of horse serum. A successful "stack" was achieved using a 4-inch diameter column. The study shows that the new method is capable of protein and enzyme separations on a scale of 10 to 100 g.

3. Low-Cost Institutional Foods From Corn

Purpose: Corn is a promising source of inexpensive food products that meet the requirements of attractiveness, nutritive quality, and general acceptability necessary for institutional feeding programs. School lunch and other public assistance programs also are in need of foods that provide maximum nutritional benefit at minimum cost.

Progress: Compositional studies of air-classified corn germ flour were completed. Several fractions separated by air were of superior nutritional quality when compared with the original flour.

A sensitive gas chromatographic method was developed for determining 3-deoxy-D-glucosone and other reactive sugar degradation intermediates in browning reactions. By an extension of this analytical method, a rapid gas chromatographic method was developed for analysis of the glycerol and triglyceride content of dry-milled corn products and vegetable oils.

A rapid, simple method based on determination of zein content of corn has been developed for evaluating the nutritional quality of protein in high-lysine corns with vitreous kernels. Dye-binding provides a rapid, economical method for monitoring loss of nutritional quality in corn or corn germ due to excessive heating during drying or processing. A high-protein concentrate rich in essential amino acids including lysine and having acceptable flavor has been extracted from defatted corn germ. The influence of concentration of SO_2 , used in wet milling corn, upon disulfide cleavage and enzyme activity during steeping was further evaluated. Automated and computerized techniques were employed to establish molecular weights of corn glutelin proteins.

An acceptable instant CSM product can be made with corn as the cereal component (see page 2). Peroxidase and lipoxidase can be inactivated in whole ground corn meal by a 2-hour indirect steam heat treatment. Storage stability studies conducted at 26° and 37° C. for 120 days indicate no off-flavors or odors, and activity of the two enzymes at zero. In fortification of corn grits with high levels of iron, the major difficulty appears to be achievement of adequate and even distribution of the enrichment mixture. Flavor and odor scores of iron-fortified sirups appeared to be satisfactory after storage for 1 year at 77° F. However, appearance of the samples ranged in color from a light yellow to an amber. In several samples, particularly those fortified with ferrous salts, there were precipitates.

The interaction of α -tocopherol or its model compound, pentamethylchromanol, with fatty acid hydroperoxide, was investigated. A number of isomeric fatty acid-tocopherol or fatty acid-pentamethylchromanol addition compounds were formed. The products indicated the addition proceeded through semiquinone and alkoxy-fatty acid free radicals. A number of other antioxidants tested did not form addition compounds.

Further modifications in the extraction procedures previously developed under contract at the University of Kentucky were necessary to achieve sufficiently complete extraction of protein both from corns with opaque (floury) and from those with vitreous (horny) endosperm. With these modifications, four opaque and two vitreous endosperm varieties were analyzed for lysine by the dinitrophenyl method. Results were in good agreement with checks by the conventional ion exchange procedure. Phenylmercuric chloride proved to have advantages over mercuric chloride as the S-H blocking agent used before treatment of samples with 2,4-dinitrophenylsulfonate.

Investigations under a grant to the University of Missouri have shown that essentially all of the phytate in the corn kernel is in the germ. All of the phytate in corn, rice and wheat, the organic phosphorus precipitated by ferric ion, is in the form of inositol hexaphosphate. A biological assay for zinc was developed using both rats and chicks and two types of basal diets--one based on casein-gelatin and one on soybean protein. A total of eight trials involving approximately 1,600 chicks was used to evaluate 12 food-stuffs. Approximately 150 growing rats also were fed the same supplements and standards diet based on soybean protein. The zinc in cereal grains as well as in oilseeds is 60-70% available to the chick. The zinc in corn germ, which has a high phytate content, is somewhat less available while that in eggs, milk, and oysters is from 80 to 100% available. In general, the rat assay gave lower availability values but the same general relationships were found.

4. Rennin From Microbial Sources

Purpose: Rennin, extracted from the stomach lining of milk-fed calves and used almost universally in cheese manufacture, is rapidly rising in price as the availability of the animal source diminishes. An alternate source for a milk-curdling agent is needed.

Progress: The Rhizopus oligosporus protease exhibits trypsinogen-kinase activity which activates trypsinogen to trypsin. Not enough data have been obtained to explain this result, but it appears to be due to a specific activity of some acid-proteases. R. oligosporus, R. chinensis, and Actinomucor elegans produce a family of closely related peptide antibiotics which contain all the commonly known amino acids and have a high content of carbohydrates. Because of their composition, these antibiotics are unusual.

Rhizomucor (Mucor pusillus) has been isolated from ground corn and animal feedlot wastes. A new species has been discovered. Conditions for obtaining zygosporos in the species R. reflexus were discovered. Seventeen of 62 R. oryzae strains formed zygosporos in matings.

At the University of Wisconsin, under grant, work on the effects of UV irradiation on spores of Aspergillus oryzae var. effusus was continued and extended to include R. oligosporus. R. oligosporus has a strange response to irradiation; it is apparently completely resistant to mutation but not to killing. Auxotrophs appear after irradiation but upon repeated retesting revert to normal types. This behavior of microorganisms is peculiar and unexpected.

5. Dry-Milling of High-Lysine Corn

Purpose: The higher nutritive value of high-lysine corn may result in its becoming the predominant corn within a few years. However, presently available strains, such as opaque-2 corn, lack the horny endosperm required to produce high-quality grits by customary dry-milling techniques. Prime products also tend to be of lower quality than those from dent corn. It is important, therefore, to determine whether conventional milling techniques can be better adapted to high-lysine corn.

Progress: Tempering opaque-2 corn without heat from 11-13% to 18-20% moisture for a conventional first temper followed by a short dehulling temper in which 5% more water was added gave satisfactory results with either a Beall degermer or the Northern Division's experimental horizontal-drum degermer. Substitution of a continuous flow vibratory sifter for a batch-type laboratory sifter in the rolls-and-grading flow for fractionating degermer stock into grits, etc. gave satisfactory results. Germ-grit separation on a gravity-table separator proved unsatisfactory and confirmed an earlier conclusion which was based on inability to use the sink-float laboratory procedure.

Interest in the multialeurone layer corn, Peru 442, has been supplanted by discovery that the South American race of maize, "coroico", is the probable source of the multialeurone character found in Peru 442. Whereas less than half of the kernel population of Peru 442 showed more than a single row of aleurone cells, all kernels of coroico have multiple aleurone. In two typical accessions the average number aleurone cell rows/kernel was 3.7. Both the aleurone content (3.4% of kernel) and the protein content of the aleurone (over 35% protein, with 4% lysine) of coroico are much higher than in ordinary corn. About 18% of the endosperm protein and over 40% of the endosperm lysine are found in the multialeurone layer of coroico. Crude lipid in the layer is about 22%.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Starch Graft Copolymers

Purpose: Markets for water-soluble high polymers as thickening or flocculating agents and as adhesives are continually expanding.

There are numerous opportunities to penetrate these markets with starch-based products derived by graft copolymerization of starch with commercially available organic monomers. Such products should effectively compete with nonagricultural synthetics in these markets.

Progress: Graft copolymers of starch and the nitric acid salt of N,N-dimethylaminoethyl methacrylate function as flocculants for silica and as retention aids for mineral-filled papers. Flocculating ability depends mainly on percent add-on and is not greatly influenced by molecular weight or frequency of grafted branches. Graft polymerization of acrylonitrile or methylacrylate onto cationic starch produces starch graft copolymers having latex-like properties. Ceric ammonium nitrate reacts with starch to form cationic complexes which are believed to be intermediates in the grafting process. Kinetic studies on grafting acrylonitrile to starch through cerium(IV) initiation showed that the ratio of weight average to number average molecular weight and the number average molecular weight of polyacrylonitrile side chains increase with time in the case of gelatinized starch. For granular starch, the ratio of weight average to number average molecular weight increases and the number average molecular weight decreases.

In research conducted under contract by General Mills Chemicals, Inc., Minneapolis, Minnesota, starch graft terpolymers containing 50% add-on of poly(acrylamide-methacryloyloxytrimethylammonium monomethyl sulfate) show considerable potential for industrial use as wet additives in papermaking, as flocculants for solids in mineral ore processing waters, and as additives for beneficiation of phosphate ores. Results from experimental paper machine trials at the Northern Division reveal that the starch graft polymers exhibit both technical and economic superiority over presently used additives for retention of fillers. Also, the terpolymers lower the level of solids in the white water much below that of presently used additives. On-site trials at certain ore processing companies show that the terpolymers are good flocculants for solids in the processing waters. A mixture of starch-polyacrylamide and hydrolyzed starch-polyacrylonitrile polymers gives excellent performance as thickeners for textile print pastes. Hundred-pound quantities of some of the starch graft polymers have been prepared for further on-site trials and industrial-scale evaluations.

Under contract research at Stanford Research Institute, Menlo Park, several new graft polymers were made on a laboratory scale by grafting ordinary unmodified starch, Amylon VII, and a 60-fluidity corn starch with selected comonomer and termonomer combinations of the following monomers: acrylic acid, acrylonitrile (AN), butadiene (BD), isoprene, and styrene (S). Graft chain content

of the products ranged from about 5 to 25% depending on the nature of the starting materials and reaction conditions. The graft polymers were screened by laboratory methods for their utility as wet-web strengtheners and as coating adhesives for paper. The most promising wet-web strengthener (an AN-BD-S graft polymer of unmodified starch) and several potential coating adhesives (AN-BD-S, AN-BD, and AN-S graft polymers of unmodified starch and Amylon VII) were prepared on a pilot-plant scale (about 1 kg.) for evaluation. This research was completed during the year.

2. Starch Xanthides as Wet-Strength Agents in Paper

Purpose: Starch xanthides, discovered at the Northern Division, are promising new products for use as additives to improve strength properties in paper. Successful commercialization of starch xanthides would be an important contribution toward meeting competitive advantages of synthetics and toward maintaining and expanding the large volume of starch products now consumed by the paper industry.

Progress: Data describing properties imparted to paper and board by starch xanthate interpolymers appear encouraging. Approximately 80 pilot-paper-machine trials were made in addition to handsheet evaluations, chemical analyses, and physical testing. Studies of interpolymer reactions of xanthate-Keymene 557 (a polyamine-polyamide-epichlorohydrin resin) indicate that a secondary valence bond (xanthate ester) is formed that links the two polymers.

New methods have been discovered for the conversion of carbohydrate C-O bonds into C-S bonds. Dithiobis(thioformate) derivatives of carbohydrates (xanthides) decompose with pyridine or photolyze with ultraviolet light to yield thio sugars. Cyclic thionocarbonates also form thio sugar on treatment with inorganic salts. Sugar xanthides react with hydrogen peroxide to form a new functional group $RO(C=S)SOH$, which is capable of initiating the polymerization of acrylic monomers.

Epoxypropyl groups have been attached to starch, cationic starch, and aminoethylated flour to give products which when employed as paper additives impart significant improvements to strength properties of the paper. Formation of polyelectrolyte complexes from cationic and anionic starches in the presence of pulp gives paper with increased strength. Starch graft copolymers have been prepared by ultraviolet irradiation of an aqueous suspension of starch containing hydrogen peroxide and acrylic monomer.

It has been determined, under grant research at Ohio State University, Columbus, that 6-aldehyde derivatives of starch, amylose, and

cellulose, prepared by photolysis of the C-6-azides, are readily characterized by degradation after mercaptolysis. Acid hydrolysis of 2,3-unsaturated amylose affords 2-(D-glycero-1,2-dihydroxyethyl) furan in 30% yield along with 3,6-anhydro-D-glucose and 6-deoxy-D-glucose. These latter two products are also obtained on acid hydrolysis of 6-O-tosylamylose. Acid hydrolysis of 6-O-2,3-di-O-tosylamylose gives D-mannose and D-glucose.

3. Cationic Starches and Flours for Paper

Purpose: The paper industry now uses a sizeable quantity of cationic starches, but much of the market can be lost to synthetic polymers if the price of cationic starches is not reduced or if performance is not improved. A dry process for manufacturing cationic starches would avoid the increasing cost of pollution control currently encountered in wet processes.

Progress: Carbamoylethyl ethers of starch on treatment with sodium hypochlorite form colloidal suspensions which are highly effective as wet-end additives for improvement of strength properties of paper. A method of making ethylenimine-reacted starches and flours by contacting the solid with vapor-laden inert gas was developed. Absorption of the ethylenimine by the solids, tested by gas chromatography, was substantially complete at solids moisture contents greater than 10%. This method should ensure a uniformly reacted product. Heating the reaction mixture after contacting increased the amount of reacted ethylenimine and improved product quality. Product quality problems encountered earlier with aminoethylated flours have been resolved and consistent high-quality derivatives can now be made on semi-commercial-scale equipment. Mill trials and extension of applicability to various paper grades with several flour substrates have been made. Exploratory experiments designed to produce products from unmodified and modified cereal flours with varying degrees of crosslinking were carried out by dry-state methods employing 0.2 to 5 weight percent of either formaldehyde or epichlorohydrin. A family of crosslinked products was obtained in which the flour protein was also crosslinked up to 98%. However, only flour that was treated with ethylenimine, neutralized, and then reacted with epichlorohydrin displayed acceptable cationic activity.

Information crucial to the successful development by an industrial company of a semicommercial process to produce high-quality cationic cereal flours (corn, wheat, and sorghum) in ton-lot quantities was provided by 716 analyses and tests on 94 lots (100 lb.) of cationic

flours. Vital information needed to judge the performance of these cationic flours in numerous machine runs at the Northern Division and in two successful commercial mill trials was also provided through a total of 208 analyses on paper and white-water samples. In continued laboratory studies on flocculation of Celite, no preferential advantage was obtained with any of the various inorganic and organic acids used in the neutralization process of cationic cereal flours. However, when the flours were treated with alkali to increase their solubility in water, their power to flocculate Celite was lost.

Studies of flow of starch slurries through pipes, under a contract with Iowa State University, Ames, indicate that the flow is non-Newtonian, but appears to conform to the power-law function. Calculation of pressure drops from this function is not too difficult. Sorption studies show that water absorbed by starch from benzyl alcohol slurries of starch behaves about in the same way as it does when absorbed from air by starch. Generalization of this conclusion would have value in industrial treatment of starches. A mixed bed of starch and glass beads or polyethylene powder has been successfully fluidized. A mixed bed can be adapted to a flow-through system for continuously treating starches or flours more efficiently.

4. Starch-Derived Chemicals for Plastics and Coatings

Purpose: Extensive opportunities exist for products derived from cereal starches and flours to share in the rapidly expanding plastics and coatings industries, thus providing benefits to agriculture through new outlets for grain.

Progress: Poor compatibility of whole corn starch with toluene diisocyanate (TDI), a monomer commonly used in urethane plastic formulations, limited our studies of the possible use of starch in such plastics. To help overcome this problem, several nonpolar groups, both cyclic and straight chain, were introduced into starch. The effects of these various groups on starch reactivity were determined by combining the starch derivatives with TDI under conditions such that reaction between unmodified starch and TDI was negligible. Octanoate, laurate, palmitate, and stearate esters of starch readily reacted with TDI when the starch molecule contained as little as 0.4 fatty groups/anhydroglucose unit (AGU). Maximum reactivity was achieved when the degree of substitution (DS) was about 0.7 to 1/AGU. At this level about 1 equivalent of TDI reacted per AGU. Acetate groups did not improve reactivity. Benzoyl esters and benzyl ethers at DS 1 were nearly equivalent to the fatty derivatives. These data show that starch can be modified to make it more compatible with TDI and suggest that any type of nonpolar radical containing an average of 6 to 8 carbons/AGU is sufficient to provide increased reaction with TDI. When the modified starches were mixed into castor oil-TDI

formulations and molded into plastics, an increase in compatibility and reactivity with TDI was evidenced by the formation of flexible, transparent plastics. Using the novel reagent of hexamethylphosphoramide solubilized lithium halides in toluene, several substituted carbohydrate sulfonates were converted to the corresponding methyl chloro-, bromo-, and iodo-glucopyranoside in excellent yield and needed short reaction times. A relationship was found between the thin-layer chromatographic behavior of some substituted methyl alpha-D-glycopyranosides and their chemical structures.

At Jadavpur University, Calcutta, India, under a PL 480 grant, the complexing reaction of calcium, strontium, and barium hydroxides with D-glucose, D-glucosamine, maltose, and amylose was extended to sucrose, cellobiose, lactose, trehalose, and amylopectin. For the mono- and disaccharides in 80% methanol-water, equimolar (1:1) adducts were formed in yields ranging from 57% to 98%, except for trehalose which gave a 45% yield with barium hydroxide. Maltose adducts were formed in highest yields: 68, 87, and 98% for Ca, Sr, and Ba hydroxides, respectively. Each interaction was examined by Job's technique and the precipitated adducts were analyzed for metal content to prove the 1:1 combining ratio. Amylose and amylopectin formed insoluble complexes in aqueous solutions with the hydroxides. Combining ratios were 2:1 anhydroglucose units per metal atom for Ca, but 1:2 for Sr and Ba hydroxides. Experiments performed with varying ratios of amylose and amylopectin showed that the yield of precipitated complex was directly proportional to the amylose content of the synthetic mixture. The 1:2 barium hydroxide-amylose complex was much less soluble than the corresponding amylopectin complex and a separation was achieved. Complexing of D-glucose with calcium chloride in nonaqueous 2-pyrrolidinone was observed.

5. Starch Reinforcement of Rubber

Purpose: The principal reinforcing agent for rubber is carbon black, which is characterized by its intense black color. Only a few expensive nonblack reinforcing agents reinforce rubber to a degree approaching carbon black. Therefore, nonblack rubbers must ordinarily be formulated with low-reinforcing fillers and have poor strength and wear properties. Recently the Northern Division discovered two new processes for reinforcement of rubber with starch. One process involves zinc starch xanthate or starch xanthide. These starch derivatives permit formulation of strong semitransparent rubbers that can be pigmented to give white or brightly colored rubber products. The second involves a crosslinked starch-resin condensation product. This product reinforces rubber as effectively as some grades of carbon black but is less expensive. Successful commercialization of these starch products as reinforcing agents for rubber would open a new market for starch potentially capable of consuming over 1 billion pounds of starch per year.

Progress: Statistically designed studies on the starch xanthate-latex masterbatching process were completed which provide a basis for selection of best masterbatch types for meeting rubber industry requirements in many end-use applications. Powdered starch-elastomers were prepared which are suitable for a variety of end-use applications. Cost estimates were obtained which show powdered starch-elastomers are similar in cost to conventional baled elastomers. Powdered starch-elastomers have been prepared by a new laboratory procedure simulating fluidized bed drying of wet starch-elastomer curd. This process avoids the hammermilling operation previously used and gives a similar particle size distribution with improved resistance to caking in storage. A new procedure has been developed for coprecipitation of alkali-starch solutions with latices by using calcium chloride. Such lime-precipitated starch-rubber masterbatches are potentially lower in cost than starch xanthide-rubber masterbatches; however, present lime-precipitated starch-rubber masterbatches give generally poorer performance than starch xanthide-rubber masterbatches.

In contract studies at B. F. Goodrich Chemical Company, Independence, Ohio, bench-scale processes have been developed for continuous coagulation of starch xanthate-SBR masterbatches of various types. Heat agglomeration and other procedures have been developed for increasing size and drainage rate of starch-rubber coprecipitated crumb. Improved procedures result in clear serums completely free of starch or latex fines and give cohesive crumbs for easy washing in cold water and for removal of water by squeezing. Properties of starch-elastomer masterbatches prepared by the improved continuous processes are the same as those of masterbatches prepared by previous batch coprecipitation procedures.

6. Biodegradable Surfactants and Detergent Builders

Purpose: The need for environmental quality control has emphasized the importance of biodegradable surfactants and the probable desirability of eliminating phosphate builders from detergents. Several types of starch derivatives have chemical structures justifying evaluation of their potential as biodegradable surfactants and builders. Successful development of starch derivatives in either or both of these applications would open an important new market for starch.

Progress: Citric and diglycolic acids were evaluated for their building effect in hard water. Sodium tripolyphosphate (STPP), sodium nitrilotriacetate (NTA), and polyitaconic anhydride, all of which exhibit good building action with linear alkyl benzene sulfonate detergents, were used to confirm the adequacy of the detergency test procedure. The sodium salts of the acids were

incorporated in the detergent formulations on a 50% weight basis. The detergent building efficiencies in hard water (300 p.p.m. hardness) were STPP, 100%; NTA, 139%; polyitaconate, 148%; citrate, 61%; and diglycolate, 72%. Values for chelation of calcium ions, reported as mg. CaCO_3 /g. of compound at pH 10 and 25° C., were STPP, 341; NTA, 321; polyitaconate, 461; citrate, 94; and diglycolate, 206.

A literature survey on surfactants that contain carbohydrate moieties was conducted. Several pounds of glycerol glycosides and ethylene glycol glycosides were prepared in the laboratory for use as base materials in the synthesis of various biodegradable surfactants and emulsifiers.

At Western Michigan University, under grant, the catalytic air oxidation of starch was extended to investigate the effect of ultra-violet light on the course of the reaction. Silver and ceric ion catalysts were utilized in the study with various additions of photosensitizer, ammonium and potassium persulfates, and hydrogen peroxide. In general, oxidized starches of low carbonyl and carboxyl contents were obtained by these procedures.

7. New Classes of High-Amylose Starch

Purpose: High-amylose corn starch is now produced commercially at a level estimated to be of the order of 30 million pounds per year. The strains of high-amylose corn used yield starch containing 50-60% and 60-70% apparent amylose. Corn breeders have for a number of years sought strains having increasingly high contents of amylose. More recently, however, interest has turned toward improvement of existing commercial strains; e.g., strains are desired that give higher yields per acre or that have higher total starch content. Research at the Northern Division is part of a cooperative program with corn breeders. Emphasis is placed on providing analyses of breeding strains and on development of basic information on amylose that contributes to increased industrial utilization of high-amylose starch.

Progress: Total starch in corn and percent amylose in starch were reported on approximately 5,500 samples submitted by breeders involved in amylomaize development. About 17% of the samples had starch with apparent amylose over 85%. More than 160 samples of amylomaize had a starch content slightly over 70% approximating that of ordinary corn (72 to 74%). Phosphorus and protein analyses of hand-isolated parts of corn kernels and histochemical studies of endosperm proteins in different varieties of maize are in progress.

Total carbohydrate analysis by phenol-sulfuric acid colorimetric method has been modified for use with a Technicon Autoanalyzer in a

system in which either dimethylsulfoxide or four molar guanidine hydrochloride is a solvent. This analysis system can effectively measure concentration gradients of 10 micrograms per milliliter. Fractionation of amylose through porous glass has been successfully demonstrated. One may now characterize starchlike materials by their elution behavior through columns of porous glass.

Small angle light scattering experiments have been applied to studies of amylose films and amylose gels at State University College of Forestry, Syracuse University, under grant. Comparison of scattering calculated by theory from geometric models with experimentally observed scattering from amylose films has led to identification of the following types of structures in amylose films: (a) Randomly oriented anisotropic rods with principal polarizability parallel to rod axis; (b) randomly oriented aggregation of rods to form a super-texture equivalent to rods with optic axis making an angle with rod axis; (c) random anisotropic texture; (d) small simple spherulites; (e) large-ringed spherulites; and (f) heterogeneous spheres imbedded in a homogeneous matrix. There is random anisotropy inside of the spheres.

At Arizona State University, Tempe, results of X-ray data and computer calculations on the structure of V-amylose are almost complete. These results show: (a) The V helix is lefthanded with six glucose residues per helix turn in both hydrate and dehydrate; (b) a 30° rotation of the helix occurs in changing from dehydrate to hydrate, and the hydrate structure has one water molecule per glucose residue located at the helix exterior; and (c) oxygen at carbon atom six was found to be in gauche-trans conformation in the hydrate. Calculations based on packing volume considerations show the upper limits in total water content of V-amylose hydrate and dehydrate to be 1.8 and 0.8 water molecule per glucose unit, respectively. Complexing molecules found in amylose-ethylenediamine and amylose dimethylsulfoxide have been proportioned between interstitial and intrahelical channels as ratios of 4:2 and 6:2, respectively, in these compounds.

Under a PL 480 grant at the University of Edinburgh, Edinburgh, Scotland, an improved apparatus for the semi-micro, differential, potentiometric titration of starch and its components with iodine has been described. A new technique of dissolving starch in dimethylsulfoxide and defatting the dissolved material has resulted in values of iodine binding capacity which are 5-15% higher than previous measurements. Some barley starches have been examined in detail and two varieties of waxy barley originating in Japan, Sumiremochi and Mochimugi, have been characterized. These two starches were essentially amylopectin-like in character. Two barley genotypes, the normal American variety, Glacier (C.I. 9686) and a mutant with a low, mean, starch granule size designated Glacier (Ac 38) were examined. Iodine titration confirmed that the mutant has a starch of high-amylose content.

8. Dialdehyde Starch-Protein Plywood Glue

Purpose: Plywood glues made with dialdehyde starch hold promise of successfully competing with presently used glues containing synthetic resins. If successfully commercialized for this use, the utilization of this starch derivative would be greatly increased.

Progress: Veneer-gluing conditions found satisfactory for fabricating Type II hardwood plywood were varied in part to study the effect on plywood glue bond quality of changes made in veneer moisture content or in glue spread level. Birch panels which met test requirements for Type II hardwood plywood were made from veneers having a moisture content range of 5 to 9%. At a veneer moisture content of 9%, a reduction in glue spread from the conventional 88 pounds per thousand square feet double glue line to 66 pounds was possible.

Soluble dried chicken blood was evaluated as an ingredient of the dialdehyde starch-protein glue for use in interior-type hardwood and softwood plywood manufacture. Yellow birch and southern yellow pine plywood panels meeting the industry's standards for bond quality were prepared using chicken blood on an equal weight basis with soy flour.

9. Lower Cost Xanthan Production Process

Purpose: Commercial consumption of xanthan gum for industrial purposes has been expanding, but demand could be increased considerably if production costs could be decreased. Approval by FDA of xanthan gum for food purposes intensifies the need to minimize production costs. Development of a continuous fermentation process is one promising approach to lowering production costs.

Progress: Single-stage continuous fermentations to produce xanthan biopolymer have been run in the pilot plant at dilution rates of 0.034 and 0.05/hr. To reduce process cost, residual glucose was maintained at the lowest value without fermentation becoming glucose limiting. Daily xanthan production rates were 0.41 and 0.60 g./hr./kg. of fermentor capacity. Overall yields, based on dextrose charged, were approximately 65% and 74%, respectively. Duration of runs was ca. six turnovers of the fermentor contents. Broth was drum dried without difficulty and water evaporation rates were approximately 12 lb./hr./sq.ft. of drum surface. Viscosity-characterization of drum-dried product reconstituted in water was 10-15% lower than the original broth. Curves have been developed relating dilution rate to fermentation viscosity and xanthan production rates. These would facilitate determining most feasible operating conditions for obtaining broth with desired viscosity.

10. L-Asparaginase Production

Purpose: L-Asparaginase has been shown to inhibit the growth of various lymphosarcomas associated with leukemia. At present, the only commercial source of the enzyme is the microorganism Escherichia coli. Alternate sources of enzyme are desirable to increase the supply of enzyme, to avoid toxicity associated with E. coli enzyme, and to provide an alternate in case of allergic reaction to E. coli enzyme. The ARS Culture Collection provides an excellent opportunity to find new and superior microbial sources of L-asparaginase.

Progress: Manipulation of media and aeration increased Erwinia aroidae asparaginase yields from 7 IU/ml. to 17.5 IU/ml. New methods enabled 100% release of enzymes from cells. Continuing improvement of purification techniques resulted in some crystalline product being formed.

11. Microbial Polysaccharides

Purpose: Colloidal substances used extensively by industry to bind water, thicken and stabilize solutions, and flocculate suspensions, traditionally comprise imported gums, extracts of seaplants, starches, and certain starch and cellulose derivatives. Research at the Northern Division showed that polysaccharides produced by certain microorganisms could not only replace traditional materials in selected applications but also provide superior performance. One of these microbial gums, xanthan gum, is now produced commercially by several U.S. and foreign companies. To the expanding industrial market for xanthan gum can be added a large new market expected to develop now that FDA has approved xanthan for use in foods. It is anticipated that microbial sources can be found for polysaccharides that have different properties from xanthan and, therefore, can find use in applications for which xanthan gum is unsuitable. Rising prices and limited supplies of imported gums, as well as expanding and changing demands by industry, make new and additional sources of gums necessary.

Progress: Conditions have been worked out for obtaining high laboratory yields of extracellular yeast mannan. Small-scale pilot-plant fermentation studies have been initiated to duplicate the laboratory results. Work with Hansenula holstii genetic variants and related yeasts has shown that mannan synthesizing capacity parallels that for phosphomannan. Certain H. holstii diploids have greatly increased capacity for polymer synthesis. Periodate oxidation studies have confirmed the earlier inference, based on concanavalin A interaction, that structurally different mannans are in hand.

Morphology and molecular interaction in aqueous solutions of eight anionic microbial polysaccharides produced in this research have been

elucidated through measurement of the magnitude of flow birefringence and extinction angles by use of an especially designed micro-Couette cell functioning over an unusually wide range of shear, and by microscopic examination of solutions and films under polarized light. Most of these polyelectrolytes show strong, ordered molecular interaction even when at rest after shear, and appear to consist of elongated birefringent forms.

Complete depolymerization of polysaccharide Y-6272 with minimal degradation of constituents, a necessity for both precise compositional analysis and ultimate isolation of the hexosaminuronic acid constituent, has been advanced through two different approaches: (1) glycosidation by MeOH or $^{14}\text{CH}_3\text{-MeOH-HCl}$ and (2) action of HNO_2 to deaminate and depolymerize N-deacetylated oligomers remaining after acid hydrolysis.

Continuing work on culture techniques of polymer-producing microorganisms resulted in isolation of two extracellular products from Xanthomonas campestris NRRL B-1459 which differ both in yield and characteristics. Improved conditions were worked out for maintenance of Hansenula holstii NRRL Y-2448. Studies with the "black" yeast NRRL Y-6272 on an improved medium show good yields and viscosities of hexosamine polymer. A pink strain of Y-6272, developed by mutation, also produces the hexosamine polymer but much less melanin.

The discovery and isolation of mating strains of the yeast Candida lipolytica opens up the possibility of breeding improved strains of this yeast for production of microbial protein for food and feed (see page 4). Sex-specific growth factors are formed by complementary mating types of the yeast Hansenula anomala. One constituent is soluble in organic solvents; the other is precipitable with ammonium sulfate. Both factors are unstable. Physiological changes that occur during meiosis and early sporulation do not interfere with the biosynthesis of either extracellular mannans or phosphomannans in diploids of the yeast H. holstii.

At Texas A and M University, under grant, several model compounds, requisite for studies planned on the role of ionogenic groups in polyelectrolyte behavior, have been prepared, purified and characterized. NMR techniques developed appear encouraging for locating the position of substituents in both the mono- and polysaccharide derivatives. A technique, believed to be generally applicable to polysaccharides, has been developed for causing clinical dextran to "crystallize" in a form never heretofore observed for this polysaccharide. A modified solvent replacement technique, generally applicable in principle to any hydrophilic biopolymer, has been developed for total removal of water for analytical or preparative purposes. The method discloses that the conventional method for moisture analysis in polysaccharides (high-vacuum desiccation under heat) removes only 56% of the water present in clinical dextran.

Under a PL 480 grant, research at the Weizman Institute of Science, Rehovoth, Israel, strains from three bacterial species have been found that produce good yields of water-soluble extracellular polysaccharides containing amino sugars in major proportions. A variable has been identified in the medium that relates to increased yields. Effective means have been established for recovering products of suitable purity and for identifying the constituents.

At the National Sugar Institute, Kanpur, India, a total of nine different aldobiuronic acids and seven aldotriuronic acids from the gums of 10 different Indian trees and shrubs have been isolated, purified, and, in most cases, identified. Amounts range from 50 milligrams to 4 grams. These rare substances are composed of various combinations of uronic acids (β -D-glucopyranosyl uronic, 4-O-methyl-D-glucopyranosyl uronic, or α -D-galactopyranosyl uronic) linked to neutral hexoses (D-galactose, D-mannose, or L-rhamnose) at carbons 2, 4, or 6. Research necessary for this accomplishment has established new information of basic value on hydrolysis of polysaccharide gums and chromatographic fractionation of sizable amounts of aldobi- and aldotriuronic acids. This research under a PL 480 grant has been completed.

Studies, under a PL 480 grant to the Polytechnicol School, Lodz, Poland, were concentrated on the metabolism of itaconic acid and on some intermediates in the tricarboxylic acid cycle in relation to stimulation of itatartaric acid synthesis. The measurement of CO₂ evolution by the Warburg technique using cell-free crude enzyme preparations was used to indicate the activity of the cultures studied. The activity of the preparations was dependent on the age of the mycelium and the medium in which it was grown. Itaconic acid was most actively metabolized by mycelium pregrown on a mineral Larsen-Hovden medium at pH 2.2. Itatartaric acid and its lactone, as well as other tricarboxylic acid cycle intermediates, were formed. Two strains converted citric acid in the presence of malonic acid solely to itatartaric acid.

C. PROTECTING FOOD SUPPLIES FROM HARMFUL MICROORGANISMS

1. Mycotoxins in Grain

Purpose: Grains are susceptible to contamination by molds that, as a result of their growth, produce toxic substances called mycotoxins. The possible occurrence of such molds and mycotoxins in grain presents a hazard both to man and to his domesticated animals. The Northern Division's research on mycotoxins is part of a broad cooperative effort involving units of the U.S. Department of Agriculture, other U.S. government agencies, various State agricultural experiment stations, and industry. Work at the Northern Division is directed towards survey of incidence of mycotoxins in cereal grains, methods of detection,

and quantitative analysis of grains for various mycotoxins; study of microorganisms to identify species and strains producing toxins and to determine growth conditions conducive to mycotoxin production; investigation of means for inhibiting mycotoxin production; and methods for detoxifying grain and derived foods containing aflatoxin.

Progress: Pure aflatoxin M_2 has been prepared by the hydrogenation of M_1 and recrystallization from acetonitrile. Conditions for dissolving and crystallizing M_1 and M_2 have been devised and molar absorptivities have been determined so solutions of M_1 and M_2 can be standardized. Their relative fluorescent intensities on silica gel plates have been established. Solvents to separate the derivatives of aflatoxins on thin-layer chromatographic plates have been developed. Quantitative standards have been prepared for distribution and sent to 15 investigators at government, university, and industrial laboratories. These standards have been used in surveys of peanuts and milk to be converted to food products and in studies on rat metabolites related to aflatoxins and on the effect of aflatoxins on rat liver enzyme systems involved in tumor formation.

A rapid method was devised for detecting aflatoxin in corn (see page 6).

2. Southern Corn Leaf Blight

Purpose: Extensive infestation of the 1970 corn crop by a mutant strain of Helminthosporium maydis, the causative agent of southern corn leaf blight, posed a potential threat to the health and economic well-being of a large segment of the U.S. population. Research was undertaken to determine whether a health hazard existed and how such blighted corn could be handled most economically, particularly in industrial applications involving nonfood uses.

Progress: Blight-damaged corn in the South appears to be more susceptible to secondary invasion by mycotoxin producers than corn in the Midwest. Secondary metabolites produced by Helminthosporium maydis are not toxic when given orally. New mycotoxins, trichotoxins A and B, and possibly tenuazonic acid have been isolated from fungi found on blighted corn.

Losses in dry and wet cleaning blighted yellow and white corn from fields heavily to severely damaged by the southern corn leaf blight organism usually ran 3 to 9% compared with 1 to 2-1/2% for the control lots, and a normal commercial average of 3 to 4%. The cleaning steps did not remove all moldy kernels. Samples of yellow corn obtained by selective hand shelling of mold-infected ears varied appreciably in bushel test weight, 100-kernel weight, fat acidity value, and contents of fat, protein, ash, and fiber depending on the degree of blight damage.

Quality of both wet- and dry-milled products from blighted corn was found to remain generally good (see page 8). Wet-milling studies on eight samples of blight-infected corn ranging in damage from very light to heavy showed that there is a lower recovery of starch and oil as the severity of infection becomes greater. Treatment of heavily infected corn with direct steam for 60 seconds resulted in a marked reduction in microbial count in all dry-milled products. Storage of infected corn at 45° C. resulted in reduction of fungi to zero in 14 days, whereas it took 15 weeks at 37° C. to accomplish this.

Yield of dry-milled prime product mix (i.e., grits, meal, and flour) correlated roughly with bushel test weight. Fat contents of products were acceptable and calculated yield of recoverable oil varied with fat content of the corn. With conventional dry and wet procedures for cleaning the blighted corn, total mold count of the products could be placed in three groups; namely, grits (usually lowest) and mixture of low-fat meal and flour, intermediate level for high-fat meal and flour blend, and highest level for composite of degermer fines, hull, and germ fractions. Grits from blighted and unblighted corn gave fairly comparable odor and flavor scores in a preliminary taste panel test.

3. Microbiology of Cereal Flours

Purpose: A variable number of microorganisms are normally associated with corn flour. Some originate in or on the corn grain, while others enter products during milling. The microbiology of corn and corn flour is growing in importance because of increasing use of corn flour in a variety of refrigerated doughs and pastries and in various types of convenience mixes. Spoilage of moist products containing corn starch, e.g. mayonnaise and salad dressing, also poses a significant problem to the food industry.

Progress: Work was concluded on the microbiological spoilage of mayonnaise and salad dressings, and the microorganisms responsible were identified. Saccharomyces bailii was isolated from two-thirds of the spoiled dressing samples, and most of the remainder were spoiled by Lactobacillus fructivorans. A study of the microflora of corn of various moisture levels stored at different low temperatures was also concluded. There was a significant increase in numbers of *Penicillia* and *Fusaria* on high-moisture corn stored at 4° and -1° C. The microbiological survey of yellow corn, corn grits, and corn flour has begun on the 1970-1971 corn crop. Samples from three mills were received during November and February and examined for mesophilic aerobic bacteria, molds, and actinomycetes.

As of January 1, 1971, the ARS Culture Collection maintained a total of 41,334 strains of molds, yeasts, bacteria, and actinomycetes. The

permanent collection contained 13,528 of these strains. The remainder are in temporary collections. During 1970, the Collection distributed 2,851 cultures of which 1,907 were sent to investigators in the United States and 944 were sent abroad. New major additions to the Collection during 1970 included more than 200 strains of mucoraceous molds isolated in cereal microbiology work; 16 mating pairs of Mucor; 24 strains of nocardiae; 17 strains of the genus Agromyces; and 47 strains of actinomycetes isolated from feedlot wastes.

A final shipment of 10 streptomycete cultures was received from the Central Drug Research Institute, Luckow, India, under a PL 480 grant now concluded. Our routine screening tests indicated that 8 out of the 10 produced antibiotics inhibitory to our four regular test organisms. Seven demonstrated activity against a gram-positive bacterium (Bacillus subtilis), 4 against a gram-negative plant pathogenic bacterium (Agrobacterium tumefaciens), 6 against a mold (Mucor ramannianus), and 3 against a yeast (Saccharomyces pastorianus). Two strains showed no activity against any of the test organisms. All of the cultures were deposited with the ARS Culture Collection Investigations for future microbiological research projects.

The emphasis, under a PL 480 grant to the University of Allahabad, Allahabad, India, has been on a study of the microbial flora of wheat and corn with some preliminary studies on rice and other cereals. The mold flora of both wheat and corn is very diverse in kinds of species present, especially Aspergilli including mycotoxin-producing forms such as Aspergillus flavus and A. ochraceus. Particularly in corn the total microbial counts have been, in some instances, exceedingly high with figures as great as 13 million/g.

D. NUTRIENT COMPOSITION

1. Nutrient Values of Cereal Grains

Purpose: Cereal grains have enjoyed long-standing importance as ingredients of breakfast foods, and their use in snack foods is expanding. Nevertheless, in view of the growing concern with health problems centering on malnutrition, obesity, and the effects of natural and synthetic toxicants, increased information is needed regarding the composition of cereal grains and their processed products. If more accurate data were available specifying the content of proteins, essential amino acids, carbohydrates, and other nutrients, and of possibly toxic natural or accidentally introduced minor components, cereal foods of maximum nutritional quality could be more easily formulated. Such foods could aid in overcoming some of the health problems of dietary origin.

Progress: Two new reagents have been developed for the determination of the total half-cystine content of proteins: (a) 2-Vinylquinoline reacts with protein sulfhydryls to yield protein-linked S-2-(2-quinolyl-ethyl)-L-cysteine (Qe-cysteine) which can then be spectrophotometrically measured to determine the total cysteine and cystine content, and (b) 4-vinylpyridine reacts with sulfhydryls of hydrolyzed proteins to form the analogous S-2-(4-pyridylethyl)-L-cysteine (Pe-cysteine) which can be determined directly by spectrophotometric analysis or via amino acid analysis. Unlike Pe-cysteine, the absorption and extinction coefficient of Qe-cysteine is unaffected by neighboring amino acids in the protein. Hence, 2-vinylquinoline is the reagent of choice for analysis of soluble proteins. 4-Vinylpyridine is preferred for use with insoluble or slightly soluble proteins. For soluble proteins, the 2-vinylquinoline method is extremely rapid, requiring only addition and removal of reagents.

The collaborative study of amino acid analysis procedures was continued and results have been analyzed statistically. The effect of mesh size, hydrolysis technique, acid-to-sample ratio and variety on hydrolysis of corn have been under investigation. Three methods of tryptophan analysis were evaluated in connection with this study.

The photooxidation of α -tocopherol with visible light was found to require the presence of a dye-sensitizer. α -Tocopherol photooxidized smoothly in methanol to isomers of 4a,5-epoxy-8a-methoxy- α -tocopherone (34%), 8a-methoxy- α -tocopherone (14%), α -tocoquinone 2,3 oxide (6%), and α -tocoquinone (less than 1%). The four geometric isomers of 4a,5-epoxy-8a-methoxy- α -tocopherone were characterized. A mechanism involving singlet oxygen is possible.

The single oxygen reactivity of α -, β -, γ -, and δ -tocopherol was determined in methanol with methylene blue as the photosensitizer. The disappearance of tocopherols was followed colorimetrically according to the Emmerie-Engel method. Of the four tocopherols, alpha- was the most reactive ($\beta = 1.4 \times 10^{-4}$ M) and delta- was the least reactive ($\beta = 13.5 \times 10^{-4}$ M). α -Tocopherol is one of the most reactive compounds to singlet oxygen reported in the literature. The relative reactivity of the tocopherols ($\alpha : \beta : \gamma : \delta = 1 : 0.50 : 0.26 : 0.10$) correlates well with vitamin E activity.

E. ALLEVIATION OF WATER POLLUTION

1. Fermentation of Cereal Grain Processing Wastes

Purpose: About 14 billion gallons of high-BOD waste water from cereal processing plants is discharged annually into surface waters where it contributes to environmental pollution. Recent laws make

alleviation of this pollution mandatory, but disposal systems to cope with pollution represent significant cost to companies. For example, treatment of all cereal wastes by the well-established activated sludge process would result in the production of a large excess of sludge which would itself cause a disposal expense. The discovery of useful polymeric products in this sludge would reduce the cost of pollution abatement.

Progress: Activated sludge from the Peoria Sewage Treatment plant was boiled in water to extract polysaccharides. Crude yields of polymeric material obtained in this manner ranged from 2.6 to 9%. After a second purification and precipitation with ethanol (3:1, v/v), the polysaccharides were hydrolyzed with dilute acid and the hydrolyzates examined by paper and gas chromatography. Glucose, mannose, and rhamnose are the major components of sludge-derived polysaccharides. Polysaccharide produced by a sewage bacterial isolate, NRRL B-1973, contained mannose and rhamnose as the main components. This bacterium gave best polymer yields in 2 days on a Casitone/glucose medium containing phosphate buffer at a pH not less than 8.0. A new component of sludge is a copolymer containing β -hydroxyvaleric and β -hydroxybutyric acids in approximately 4:1 ratio. It melts at 100-105° C., and its infrared spectrum differs from that of poly- β -hydroxybutyrate. It has been isolated in 2.2% yield (dry weight basis), and evidence has been obtained for its production by several microbial species isolated from activated sludge.

2. Animal Waste Disposal

Purpose: The Northern Division has recently undertaken a program of research to find new economic methods for disposing of animal wastes. Such wastes present a pollution hazard of great magnitude and growing importance, particularly in view of the increasing number of feedlots containing large numbers of animals. The Northern Division's program includes such approaches as photochemical degradation of animal wastes; enzymatic conversion of wastes to feeds or fertilizers; isolation of a high-protein feed product from the liquid fraction of waste; and utilization of this liquid fraction as a fermentation medium for production of industrial chemicals.

Progress: Although the program has been active for only a few weeks, a significant advance has been made in development of an improved method for determination of insoluble carbohydrates in animal waste (see page 10).

Under contract, the University of Denver, Denver, Colorado, is conducting detailed compositional studies on typical animal wastes, and the Hamilton-Standard Company, Windsor Locks, Connecticut, is investigating production of power fuels by fermentation of these wastes.

F. MISCELLANEOUS

1. Japanese Beetle Control

Purpose: Control of the Japanese beetle by a selective biological insecticide, harmless to man and other animals and nonproductive of environmental pollution, is possible with spores of Bacillus popilliae, causative agent of milky disease in Japanese beetles. Present production of spores from infected grubs is expensive and makes extensive use of spores impractical. The Northern Division is seeking an inexpensive, commercially feasible, fermentative process for large-scale production of B. popilliae spores.

Progress: For the first time, B. popilliae was cultured continuously in a chemostat. Steady-state populations of one billion vegetative cells/ml. were obtained in a medium containing a low concentration of yeast extract. This population is significantly higher than that obtained in batch culture (500 million cells/ml.) in a richer medium. Metabolic studies of B. popilliae in diseased larvae revealed an active tricarboxylic acid (TCA) cycle with greatest activity during the early phases of disease. TCA cycle activity of B. popilliae outside the insect was compared to that of other bacteria associated with insects. B. popilliae and B. lentimorbus cells that do not sporulate have no TCA cycle activity whereas B. thuringiensis, B. alvei, and B. larvae in transition from vegetative growth to sporulation exhibited a TCA cycle.

Research, under contract at Michigan State University, revealed that unlike results with other aerobic sporeformers, there was no correlation in B. popilliae between acetate oxidation and sporogenesis. Certain strains of B. popilliae have limited ability to oxidize acetate; others do not. When the ability is present, older cells oxidize acetate more effectively than young. Strains which can oxidize acetate grow, and slowly but completely utilize α -methylmannoside. Strains which do not oxidize acetate do not grow on α -methylmannoside.

Under grant research at Baylor University, the cytology of ascosporogenesis in Hansenula wingei now has been essentially established. Conjugating cells develop protuberances through which union is achieved. Once cytoplasmic communion is established by modification and then dissolution of cell wall, the contents of the donor cell including the elongate flexible nucleus are emptied into the recipient cell. When passage is complete, the conjugation bridge is plugged by unique dense material. Nuclei of donor and recipient appear to exchange material through juxtaposed nuclear pores rather than by fusion.

Publications and Patents

1. Milling Artificially Dried Corn

Anderson, R. A. 1970. Corn wet milling industry. In "Corn: Culture, Processing, Products," ed. G. E. Inglett, chap. 9, pp. 151-170. Westport, Conn.

Blinč, M., Cimerman, A., Pertot, E., and Stucin, D. (Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia). 1970. Zur genaueren kenntnis durch temperatureinfluß modifizierter wachsmais- und amylosestärke. /Contribution on waxy maize starch and high amylose starch modified under the influence of temperature./ Staerke 22(6), pp. 181-188.*

Brekke, O. L. 1970. Corn dry milling industry. In "Corn: Culture, Processing, Products," ed. G. E. Inglett, chap. 14, pp. 262-291. Westport, Conn.

Hammerle, J. R., and Mohsenin, N. N. (Pennsylvania State University, University Park, Pennsylvania). 1970. Tensile relaxation modulus of corn horny endosperm as a function of time, temperature and moisture content. Trans. ASAE 13(3), pp. 372-375.

Hammerle, J. R.,¹ White, R. K.,² and Mohsenin, N. N.³ (¹North Carolina State University, Raleigh, North Carolina; ²Ohio State University, Columbus, Ohio; ³Pennsylvania State University, University Park, Pennsylvania). 1971. The rheological properties of corn horny endosperm. Cereal Sci. Today 16(2), pp. 60-64, 66-68, 72.

Inglett, G. E. 1970. Carbohydrate and protein interactions in cereal processing and products. Proc. Joint Symp. on Carbohydrate/Protein Interactions, Carbohydrate-Oilseeds Divisions, American Association of Cereal Chemists, Excelsior Springs, Missouri, June 22-23.

Inglett, G. E. 1970. Corn in perspective. In "Corn: Culture, Processing, Products," ed. G. E. Inglett, chap. 1, pp. 1-5. Westport, Conn.

Inglett, G. E. 1970. Kernel structure, composition, and quality. In "Corn: Culture, Processing, Products," ed. G. E. Inglett, chap. 7, pp. 123-137. Westport, Conn.

*Research supported by PL 480 funds.

- Inglett, G. E. 1970. Food uses of corn around the world. In "Corn: Culture, Processing, Products," ed. G. E. Inglett, chap. 8, pp. 138-150. Westport, Conn.
- Inglett, G. E. 1970. Fermentation and distilling industries. In "Corn: Culture, Processing, Products," ed. G. E. Inglett, chap. 16, pp. 307-313. Westport, Conn.
- Khoo, U., and Wolf, M. J. 1970. Origin and development of protein granules in maize endosperm. Amer. J. Bot. 57(9), pp. 1042-1050.
- Moore, C. A.,¹ and Dwoskin, P. B.² (¹USDA Marketing Economics Division, Peoria, Illinois; ²USDA Marketing Economics Division, Washington, D.C.). 1970. Economics: Production and marketing. In "Corn: Culture, Processing, Products," ed. G. E. Inglett, chap. 6, pp. 84-122. Westport, Conn.
- Simpson, T. D. 1970. X-ray diffraction of ethylenediamine-amylose complex. Biopolymers 9(9), pp. 1039-1047.
- Stephens, H. L., Roberts, R. W., Reed, T. F., and Murphy, R. J. (Institute of Polymer Science, University of Akron, Akron, Ohio). 1971. Starch-elastomer masterbatches. Preparation, properties, and process design. Ind. Eng. Chem., Prod. Res. Develop. 10(1), pp. 84-92.
- Takagi, M., Mizutani, M., Nishio, T., and Ono, S. (University of Osaka Prefecture, Sakai, Japan). 1970. Determination of carbonyl groups in starches by polarographic techniques using O-phenylenediamine. V. Reactions of periodate oxidized starches with O-phenylenediamine. Staerke 22(5), pp. 158-161.*
- Wen, P. R., and Mohsenin, N. N. (Pennsylvania State University, University Park, Pennsylvania). 1970. Measurement of dynamic viscoelastic properties of corn horny endosperm. J. Materials 5(4), pp. 856-867.
- Wolf, M. J., and Khoo, U. 1970. Mature cereal grain endosperm: Rapid glass knife sectioning for examination of proteins. Stain Technol. 45(6), pp. 277-283.
- Wolf, M. J., Melvin, E. H., Garcia, W. J., Dimler, R. J., and Kwolek, W. F.¹ (¹USDA Biometrical Services, Peoria, Illinois). 1970. Amylose determination in dimethyl sulfoxide extracts of maize. Cereal Chem. 47(4), pp. 437-446.

*Research supported by PL 480 funds.

2. Conversion of Starch to Sugars and Sirups

Boyer, E. W., and Hartman, P. A. (Iowa State University, Ames, Iowa). 1971. Extracellular transglucosylase and α -amylase of Streptococcus equinus. J. Bacteriol. 106(2), pp. 561-570.

Dick, W. E., Jr., Weisleder, D., and Hodge, J. E. 1971. A synthesis of methyl α -maltoside. Carbohydr. Res. 18(1), pp. 115-123.

El-Dash, A. A., and Hodge, J. E. 1971. Determination of 3-deoxy-D-erythro-hexosulose (3-deoxy-D-glucosone) and other degradation products of sugars by borohydride reduction and gas-liquid chromatography. Carbohydr. Res. 18(2), pp. 259-267.

Good, W. A., and Hartman, P. A. (Iowa State University, Ames, Iowa). 1970. Properties of the amylase from Halobacterium halobium. J. Bacteriol. 104(1), pp. 601-603.

Lakshmanan, C. M., and Hoelscher, H. E. (University of Pittsburgh, Pittsburgh, Pennsylvania). 1970. Production of levoglucosan by pyrolysis of carbohydrates. Pyrolysis in hot inert gas stream. Ind. Eng. Chem., Prod. Res. Develop. 9(1), pp. 57-59. Also published in Staerke 22(8), pp. 261-264.

Lehrfeld, J., and Goodwin, J. C. 1970. A 1:1 adduct of methyl 4,6-O-benzylidene- β -D-glucopyranoside and pyridinium chloride. Carbohydr. Res. 14(3), pp. 412-414.

Lineback, D. R., and Baumann, W. E. (University of Nebraska, Lincoln, Nebraska). 1970. Properties of a glucoamylase from Aspergillus phoenicis. Carbohydr. Res. 14(3), pp. 341-353.

Nakamura, L. K. 1970. Substrate inhibition of transglucosyl-amylase by maltose. J. Bacteriol. 104(1), pp. 69-73.

Nakamura, L. K. 1970. Influence of the acceptor during transglucosylation by transglucosylamylase of Candida tropicalis. Can. J. Biochem. 48(11), pp. 1260-1267.

Robyt, J. F., Chittenden, C. G., and Lee, C. T. (Iowa State University, Ames, Iowa). 1971. Structure and function of amylases. I. The subunit structure of porcine pancreatic α -amylase. Arch. Biochem. Biophys. 144(1), pp. 160-167.

Robyt, J. F., and French, D. (Iowa State University, Ames, Iowa). 1970. The action pattern of porcine pancreatic α -amylase in relationship to the substrate binding site of the enzyme. J. Biol. Chem. 245(15), pp. 3917-3927.

Russell, I. J., and Lineback, D. R. (University of Nebraska, Lincoln, Nebraska). 1970. Incorporation of ^{14}C into the carbohydrate moieties of a fungal glucoamylase. Carbohydr. Res. 15(1), pp. 123-135.

Smiley, K. L. 1971. Continuous conversion of starch to glucose with immobilized glucoamylase. Biotechnol. Bioeng. 13(2), pp. 309-317.

Smiley, K. L., Hensley, D. E., Smiley, M. J., and Gasdorf, H. J. 1971. Kinetic patterns of glucoamylase isozymes isolated from Aspergillus species. Arch. Biochem. Biophys. 144(2), pp. 694-699.

Strandberg, G. W., and Smiley, K. L. 1971. Free and immobilized glucose isomerase from Streptomyces phaeochromogenes. Appl. Microbiol. 21(4), pp. 588-593.

Thoma, J. A., Brothers, C., and Spradlin, J. (University of Arkansas, Fayetteville, Arkansas). 1970. Subsite mapping of enzymes. Studies on Bacillus subtilis amylase. Biochemistry 9(8), pp. 1768-1775.

Tyler, D. D., and Nakamura, L. K. 1971. Conditions for production of 3-ketomaltose from Agrobacterium tumefaciens. Appl. Microbiol. 21(2), pp. 175-180.

3. Low-Cost Institutional Foods from Corn

Anderson, R. A., Pfeifer, V. F., Bookwalter, G. N., and Griffin, E. L., Jr. 1971. Instant CSM food blends for worldwide feeding. Cereal Sci. Today 16(1), pp. 5-8, 11.

Blessin, C. W., Inglett, G. E., Cavins, J. F., and Deatherage, W. L. 1970. Lysine fortification of dent corn. Cereal Sci. Today 15(11), pp. 375-377, 394.

Bookwalter, G. N., Conway, H. F., and Griffin, E. L., Jr. May 18, 1971. Extruder-cooked cereal endosperm particles and instant beverage mixes comprising the same. U.S. Patent 3,579,352.

- Conway, H. F. 1971. Extrusion cooking of cereals and soybeans-- Parts I and II. Food Prod. Develop. 5(2), pp. 27, 29, 31; 5(3), pp. 14, 16, 18, 22.
- Gardner, H. W. 1970. Sequential enzymes of linoleic acid oxidation in corn germ: Lipoyxygenase and linoleate hydroperoxide isomerase. J. Lipid Res. 11(4), pp. 311-321.
- Gardner, H. W., and Inglett, G. E. 1971. Food products from corn germ: Enzyme activity and oil stability. J. Food Sci. 36(4), pp. 645-648.
- Gardner, H. W., Inglett, G. E., Deatherage, W. L., Kwolek, W. F.,¹ and Anderson, R. A. (1USDA Biometrical Services, Peoria, Illinois). 1971. Food products from corn germ: Evaluation as a food supplement after roll-cooking. J. Food Sci. 36(4), pp. 640-644.
- Gardner, H. W., and Weisleder, D. 1970. Lipoyxygenase from Zea mays: 9-D-Hydroperoxy-trans-10,cis-12-octadecadienoic acid from linoleic acid. Lipids 5(8), pp. 678-683.
- Inglett, G. E. 1970. Corn and sorghum proteins. Proc. 25th Annual Corn and Sorghum Research Conf., sponsored by American Seed Trade Association at Chicago, Illinois, eds. John I. Sutherland and Robert J. Falasca, pp. 67-78. Washington, D.C.
- Mills, F. D., Baker, B. G., and Hodge, J. E. 1970. Thermal degradation of 1-deoxy-1-piperidino-D-fructose. Carbohydr. Res. 15(2), pp. 205-213.
- Nielsen, H. C., Paulis, J. W., James, C., and Wall, J. S. 1970. Extraction and structure studies on corn glutelin proteins. Cereal Chem. 47(5), 501-512.
- Vojnovich, C., and Pfeifer, V. F. 1970. Stability of ascorbic acid in blends with wheat flour, CSM, and infant cereals. Cereal Sci. Today 15(9), pp. 317-322.
- Vojnovich, C., Pfeifer, V. F., and Griffin, E. L., Jr. 1970. Reducing microbial populations in dry-milled corn products. Cereal Sci. Today 15(12), pp. 401-404, 406-407, 411.
- Wall, J. S., Cavins, J. C., James, C., and Inglett, G. E. 1971. An economic evaluation of hominy feed. Feedstuffs 43(18), pp. 18, 20, 22.

4. Rennin from Microbial Sources

Wang, H. L., and Hesseltine, C. W. 1970. Sufu and lao-chao. J. Agr. Food Chem. 18(4), pp. 572-575.

Wang, H. L., and Hesseltine, C. W. 1970. Multiple forms of Rhizopus oligosporus protease. Arch. Biochem. Biophys. 140(2), pp. 459-463.

Wang, H. L., and Hesseltine, C. W. 1970. Oriental fermented foods. Part 1 (middle section). Seminar on Protein Food Promotion, held at The Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand, November 22-December 1.

5. Dry-Milling of High-Lysine Corn

None.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Starch Graft Copolymers

Fanta, G. F., Burr, R. C., Russell, C. R., and Rist, C. E. 1970. Graft copolymers of starch and poly(2-hydroxy-3-methacryloyloxy-propyltrimethyl ammonium chloride). Preparation and testing as flocculating agents. J. Appl. Polym. Sci. 14(10), pp. 2601-2609.

Gugliemelli, L. A., Weaver, M. O., Russell, C. R., and Rist, C. E. 1971. Molecular weight distribution of the polyacrylonitrile moiety of starch-polyacrylonitrile graft copolymers. J. Polym. Sci., Part B, 9(2), pp. 151-155.

2. Starch Xanthides as Wet-Strength Agents in Paper

Clode, D. M., and Horton, D. (Ohio State University, Columbus, Ohio). 1970. Photolysis of secondary azides of sugars. Carbohydr. Res. 14(3), pp. 405-408.

Doane, W. M., and Shasha, B. S. Sept. 15, 1970. Production of bis(1,2:5,6-O-isopropylidene-3-O-thiocarbonyl- α -D-glucofuranose) disulfide and paper containing same. U.S. Patent 3,528,880.

Ernst, J. O., Ernst, A. J., Hofreiter, B. T., Kwolek, W. F.,¹ and Hamerstrand, G. E. (¹USDA Biometrical Services, Peoria, Illinois). 1971. Maintenance of validity in physical tests of paper. Tappi 54(4), pp. 550-552.

Horton, D., and Tindall, C. G., Jr. (Ohio State University, Columbus, Ohio). 1970. Methylene-insertion reactions with unsaturated sugars. Synthesis of 4-C-cyclopropyl-D-ribo-tetrofuranose derivatives. Carbohyd. Res. 15(2), pp. 215-232.

Rankin, J. C., and Russell, C. R. July 28, 1970. Acidified ethylenimine modified cereal flours. U.S. Patent 3,522,238.

Shasha, B. S., and Doane, W. M. 1971. Chlorothioformates of sugars from the corresponding chloromethylsulfenyl chloride derivatives. Carbohyd. Res. 16(1), pp. 145-152.

Shasha, B. S., Doane, W. M., and Russell, C. R. 1971. Sugars with a new functional group: Hydroxythio(thiocarbonate). Carbohyd. Res. 18(2), pp. 251-257.

Shasha, B. S., Doane, W. M., Russell, C. R., and Rist, C. E. 1971. Reactions of a sugar chlorothioformate. Carbohyd. Res. 17(2), pp. 444-448.

Shasha, B. S., Doane, W. M., and Stout, E. I. Oct. 27, 1970. Carbohydrate trans-fused cyclic carbonate wet-end additives for paper. U.S. Patent 3,536,579.

Smith, H. E., Gordon, S. H., and Katz, H. C. Nov. 24, 1970. Wet strength paper comprising starch carbamoylethyl ethers. U.S. Patent 3,542,644.

Trimmell, D., Doane, W. M., Russell, C. R., and Rist, C. E. 1971. Thio sugars by isomerization of carbohydrate thionocarbonates. Carbohyd. Res. 17(2), pp. 319-326.

Williams, W. L., and Hofreiter, B. T. 1970. Computerized maintenance for a research paper machine. Paper Trade J. 154(32), pp. 39-40.

Wing, R. E., Doane, W. M., and Rist, C. E. 1970. Sugars with reactive substituents: Preparation of several epoxypropyl-derivatized sugars. Carbohyd. Res. 14(2), pp. 267-271.

3. Cationic Starches and Flours for Paper

Hanson, T. P.,¹ Cramer, W. D.,¹ Abraham, W. H.,¹ and Lancaster, E. B. (¹Iowa State University, Ames, Iowa). 1971. Rates of water-vapor absorption in granular cornstarch. Chem. Eng. Progr. Symp. Ser. 67(108), pp. 35-39.

Lancaster, E. B. 1971. Specific volume and flow of corn grits under pressure. Chem. Eng. Progr. Symp. Ser. 67(108), pp. 30-34.

Russell, C. R. 1970. Starch and flour products in paper and rubber. Rpt. Sixth Nat. Conf. Wheat Util. Res., held at Oakland, California, November 5-7, 1969. West. Util. Res. Develop. Div., U.S. Agr. Res. Serv., ARS 74-54, pp. 122-136.

Smith, H. E., Gordon, S. H., Russell, C. R., and Rist, C. E. 1970. Wet- and dry-strength agent for paper derived from carbamoyl ethyl starch. Tappi 53(9), pp. 1704-1708.

Wilham, C. A., Mehlretter, C. L., and McGuire, T. A. 1970. Cationic fatty acid derivatives aid retention of starch and pigment color in cellulosic pulp. J. Amer. Oil Chem. Soc. 47(8), p. 304.

Wing, R. E., Doane, W. M., and Rist, C. E. 1971. Epoxypropyl starch as a paper additive. Preparation and evaluation. Tappi 54(2), pp. 222-223.

4. Starch-Derived Chemicals for Plastics and Coatings

BeMiller, J. N., Collins, C. L., Doyle, E. R., and Wing, R. E. (Southern Illinois University, Carbondale, Illinois). 1971. Anomeric methyl 4-O-alkyl-D-glucopyranosides. Carbohyd. Res. 16(2), pp. 480-485.

BeMiller, J. N., and Wing, R. E. (Southern Illinois University, Carbondale, Illinois). 1968. Methyl terminal-4-O-methylmalto-oligosaccharides. Carbohyd. Res. 6(2), pp. 197-206.

BeMiller, J. N., Wing, R. E., and Meyers, C. Y. (Southern Illinois University, Carbondale, Illinois). 1968. The specific debenzyl-ation of alkylated carbohydrates via bromination-hydrolysis. J. Org. Chem. 33(11), pp. 4292-4294.

Entlicher, G., and BeMiller, J. N. (Southern Illinois University, Carbondale, Illinois). 1971. Methanolysis of amylose triesters and related compounds. Carbohyd. Res. 16(2), pp. 363-374.

Lakshmanan, C. M., Gal-Or, B., and Hoelscher, H. E. (University of Pittsburgh, Pittsburgh, Pennsylvania). 1970. Production of levoglucosan by pyrolysis of carbohydrates. Staerke 22(7), pp. 221-227.

Maher, G. G., Douglas, J. A., Russell, C. R., and Rist, C. E. 1970. Crosslinking of starch xanthate. II. Reaction with polyacrolein. J. Polym. Sci., Part A-1, 8(7), pp. 1637-1645.

Maher, G.G., Junker, M.L., Douglas, J.A., Russell, C.R., and Rist, C.E.
1970. Crosslinking of starch xanthate. III. Reaction with
low-molecular-weight polyamines. *Staerke* 22(8), pp. 249-255.

Rist, C. E. 1970. Starch products for plastics and flocculants.
1970. Rpt. Sixth Nat. Conf. Wheat Util. Res., held at Oakland,
California, November 5-7, 1969. West. Util. Res. Develop. Div.,
U.S. Agr. Res. Serv., ARS 74-54, pp. 137-143.

Sinclair, H. B. 1970. Displacement of carbohydrate sulfonates
with halide ions of toluene-solubilized halides. *Carbohyd.*
Res. 15(1), pp. 147-153.

Sleeter, R. T., and Sinclair, H. B. 1970. Thin-layer chromatography
of substituted methyl β -maltosides. *J. Chromatogr.* 49(3),
pp. 543-545.

Sleeter, R. T., and Sinclair, H. B. 1970. Routes to substituted
methyl β -maltosides. *J. Org. Chem.* 35(11), pp. 3804-3807.

Srivastava, H. C., Parmar, R. S., and Dave, G. B. (Ahmedabad
Textile Industry's Research Association, Ahmedabad, India).
1970. Studies on dextrinization. Part I. Pyrodextrinization
of corn starch in the absence of any added catalyst. *Staerke*
22(2), pp. 49-54.*

Srivastava, H. C., Ramalingam, K. V., and Doshi, N. M. (Ahmedabad
Textile Industry's Research Association, Ahmedabad, India). 1969.
Distribution of hydroxyethyl groups in commercial hydroxyethyl
starch. Part II. *Staerke* 21(7), pp. 181-183.*

5. Starch Reinforcement of Rubber

[B]uchanan, R. A. 1971. Powdered elastomers. Starch xanthide
RF encased FR-N600 (NBR). North Market. Nutr. Res. Div.,
U.S. Agr. Res. Serv., CA-71-32, 3 pp. [P]rocessed/

[B]uchanan, R. A. 1971. Powdered elastomers. Zinc starch
xanthate RF encased SBR 1511. North Market. Nutr. Res. Div.,
U.S. Agr. Res. Serv., CA-71-33, 3 pp. [P]rocessed/

[B]uchanan, R. A. 1971. Zinc starch xanthate encased SBR 1713.
North Market. Nutr. Res. Div., U.S. Agr. Res. Serv., CA-71-34,
3 pp. [P]rocessed/

*Research supported by PL 480 funds.

Douglas, J. A., and Maher, G. G. Nov. 24, 1970. Starch poly-ethylenimino thiourethane reinforced rubbers. U.S. Patent 3,542,708.

6. Biodegradable Surfactants and Detergent Builders

McGuire, T. A., and Mehlretter, C. L. 1971. Chemical process for making dialdehyde starch. Staerke 23(2), pp. 42-45.

McGuire, T. A., and Mehlretter, C. L. Jan. 26, 1971. Process for the chlorohydrination of allytrimethylammonium chloride. U.S. Patent 3,558,501.

Mehlretter, C. L. Oct. 27, 1970. Calcium sequestration in highly alkaline medium. U.S. Patent 3,536,630.

Wilham, C. A., McGuire, T. A., Mark, A. M., and Mehlretter, C. L. 1970. Starch-derived polyelectrolytes as builders in heavy duty detergent formulations. J. Amer. Oil Chem. Soc. 47(12), pp. 522-524.

7. New Classes of High-Amylose Starch

Banks, W.,¹ Greenwood, C. T.,¹ and Walker, J. T.² (¹University of Edinburgh, Edinburgh, Scotland; ²Scottish Plant Breeding Station, Pentlandsfield, Scotland). 1970. Studies on the starches of barley genotypes: The waxy starch. Staerke 22(5), pp. 149-152.*

Dintzis, F. R., Babcock, G. E., and Tobin, R. 1971. Behavior of amylose in ethylenediamine and formamide. Carbohyd. Res. 17(1), pp. 216-219.

Dintzis, F. R., Tobin, R., and Babcock, G. E. 1971. Amylose optical rotation in some mixed solvent systems. Biopolymers 10(2), pp. 379-389.

Mark, A. M., and Mehlretter, C. L. Dec. 22, 1970. Method for the preparation of amylose acetate dispersions. U.S. Patent 3,549,619.

Mark, A. M., and Mehlretter, C. L. Jan. 5, 1971. High-amylose starch acetate. U.S. Patent 3,553,196.

*Research supported by PL 480 funds.

8. Dialdehyde Starch-Protein Plywood Glue

Van Cleve, J. W. 1971. Improved preparation of methyl 4,6-O-benzylidene- α -D-glucopyranoside. Carbohyd. Res. 17(2), pp. 461-464.

Weakley, F. B., Roth, W. B., Mehlretter, C. L., and Rist, C. E. 1971. Protein-dialdehyde starch glue for birch type II plywood. Staerke 23(2), pp. 58-62.

Wilham, C. A., McGuire, T. A., and Mehlretter, C. L. 1971. Hydrolysis of dialdehyde starch with sulfurous acid. Staerke 23(6), pp. 201-203.

9. Lower Cost Xanthan Production Process

Moraine, R. A., and Rogovin, P. 1971. Xanthan biopolymer production at increased concentration by pH control. Biotechnol. Bioeng. 13(3), pp. 381-391.

10. L-Asparaginase Production

Peterson, R. E., and Ciegler, A. June 29, 1971. Production of L-asparaginase. U.S. Patent 3,589,982.

11. Microbial Polysaccharides

Bajpai, K. S., Chandrasekharan, V., Mukherjee, S., and Shrivastava, A. N. (National Sugar Institute, Kanpur, India). 1970. Infrared absorption spectra of aldobiouronic acids obtained from natural plant gums and mucilages. Carbohyd. Res. 14(2), pp. 259-262.*

Cadmus, M. C., Bagby, M. O., Burton, K. A., and Wolff, I. A. Feb. 23, 1971. Nitrogen source for improved productions of microbial polysaccharides. U.S. Patent 3,565,763.

Heidelberger, M.,¹ and Slodki, M. E. (¹New York University Medical Center, New York). 1970. Predicted and unpredicted cross-reactions of an acetylphosphogalactan of Sporobolomyces yeast. Part II. J. Exp. Med. 132(6), pp. 1105-1106.

Herman, A. I. 1970. Interspecies sex-specific growth responses in Kluyveromyces. Antonie van Leeuwenhoek; J. Microbiol. Serol. 36(3), pp. 421-425.

*Research supported by PL 480 funds.

- Pasika, W. M., and West, A. C. III. (Texas A and M University, College Station, Texas). 1971. Quantitative determination of water in a hydrophilic macromolecular sample (dextran) by gas chromatography. *Anal. Chem.* 43(2), pp. 275-276.
- Pittsley, J. E., Bair, L. R., Sloneker, J. H., and Jeanes, A. 1971. Microapparatus for studying flow birefringence. *Rev. Sci. Instrum.* 42(6), pp. 895-896.
- Stock, D. A., and Black, S. H. (Baylor University College of Medicine, Houston, Texas). 1970. Meiosis in Hansenula holstii and H. wingei. *J. Gen. Microbiol.* 64, pp. 365-372.
- Wickerham, L. J. 1970. Genus 1. Citeromyces Santa Maria. In "The Yeasts. A Taxonomic Study," ed. J. Lodder, second rev. and enl. ed., chap. 4, pp. 121-127. Amsterdam.
- Wickerham, L. J. 1970. Genus 7. Hansenula H. et P. Sydow. In "The Yeasts. A Taxonomic Study," ed. J. Lodder, second rev. and enl. ed., chap. 4, pp. 226-315. Amsterdam.
- Wickerham, L. J. 1970. Genus 14. Pachysolen Boidin et Adzet. In "The Yeasts. A Taxonomic Study," ed. J. Lodder, second rev. and enl. ed., chap. 4, pp. 448-454. Amsterdam.
- Wickerham, L. J. 1970. Correlation of deoxyribonucleic acid base percentages and taxonomic characteristics in Hansenula. In "Recent Trends in Yeast Research," ed. D. G. Ahearn, chap. 2, pp. 31-47. Atlanta.
- Yamada, K., Minoda, Y., Kodama, T., and Kotera, U. (University of Tokyo, Tokyo, Japan). June 15, 1971. Novel process of producing L(+)- tartaric acid. U.S. Patent 3,585,109.*

C. PROTECTING FOOD SUPPLIES FROM HARMFUL MICROORGANISMS

1. Mycotoxins in Grain

- Agrawal, A. K., Mehrotra, S., Mondal, H., Trivedi, P. D., Sultana, S., Arif, A. J., Jaffri, B. J., Chandra, B., and Srivastava, O. P. (Central Drug Research Institute, Lucknow, India). 1968. Isolation of actinomycetes from different soil types of Uttar Pradesh and their antagonistic properties. *Indian J. Microbiol.* 8(4), pp. 225-232.*

*Research supported by PL 480 funds.

- Burmeister, H. R., and Leistner, L.¹ (¹Institut für Bakteriologie und Histologie der Bundesanstalt für Fleischforschung, Kulmbach, Germany). 1970. Aflatoxinbildung in fleischwaren. [Aflatoxin formation in meats. In German. English summary.] Die Fleischwirtschaft 50(5), p. 685.
- Ciegler, A., and Hou, C. T. 1970. Isolation of viridicatin from Penicillium palitans. Arch. Mikrobiol. 73(3), pp. 261-267.
- Ciegler, A., and Kurtzman, C. P. 1970. Fluorodensitometric assay of penicillic acid. J. Chromatogr. 51(3), pp. 511-516.
- Ciegler, A., and Kurtzman, C. P. 1970. Penicillic acid production by blue-eye fungi on various agricultural commodities. Appl. Microbiol. 20(5), pp. 761-764.
- Ciegler, A., and Pitt, J. I. 1970. Survey of the genus Penicillium for tremorgenic toxin production. Mycopathol. Mycol. Appl. 42(1-2), pp. 119-124.
- Detroy, R. W., and Hesseltine, C. W. 1970. Aflatoxicol: Structure of a new transformation product of aflatoxin B₁. Can. J. Biochem. 48(7), pp. 830-832.
- Detroy, R. W., and Ciegler, A. 1971. Aflatoxin biosynthesis in Aspergillus parasiticus: Effect of methionine analogs. Can. J. Microbiol. 17(5), pp. 569-574.
- Detroy, R. W., and Ciegler, A. 1971. Induction of yeastlike development in Aspergillus parasiticus. J. Gen. Microbiol. 65(Pt. 3), pp. 259-264.
- Detroy, R. W., and Hesseltine, C. W. 1970. Secondary biosynthesis of aflatoxin B₁ in Aspergillus parasiticus. Arch. Biochem. Biophys. 16(10), pp. 959-963.
- Hesseltine, C. W., Shotwell, O. L., Smith, M., Ellis, J. J., Vandegraft, E., and Shannon, G. 1970. Production of various aflatoxins by strains of the Aspergillus flavus series. In M. Herzberg, ed., Proc. First U.S.-Japan Conf. Toxic Microorganisms, Honolulu, Hawaii, October 7-10, 1968, pp. 202-210. Unnumb. Pub., U.S. Dept. of Interior and UJNR Panels on Toxic Microorganisms. Washington, D.C.
- Hou, C. T., Ciegler, A., and Hesseltine, C. W. 1970. Tremorgenic toxins from penicillia. I. Colorimetric determination of tremortins A and B. Anal. Biochem. 37(2), pp. 422-428.

- Hou, C. T., Ciegler, A., and Hesselstine, C. W. 1971. Tremorgenic toxins from penicillia. II. A new tremorgenic toxin, tremortin B, from Penicillium palitans. Can. J. Microbiol. 17(5), pp. 599-603.
- Hou, C. T., Ciegler, A., and Hesselstine, C. W. 1971. Tremorgenic toxins from penicillia. III. Tremortin production by Penicillium species on various agricultural commodities. Appl. Microbiol. 21(6), pp. 1101-1103.
- Kurtzman, C. P., and Ciegler, A. 1970. Mycotoxin from a blue-eye mold of corn. Appl. Microbiol. 20(2), pp. 204-207.
- Lillehoj, E. B., and Ciegler, A. 1970. Aflatoxin B₁ effect on enzyme biosynthesis in Bacillus cereus and Bacillus licheniformis. Can. J. Microbiol. 16(11), pp. 1059-1065.
- Lillehoj, E. B., and Ciegler, A. 1970. Aflatoxin B₁ induction of lysogenic bacteria. Appl. Microbiol. 20(5), pp. 782-785.
- Lindenfelser, L. A., and Ciegler, A. 1970. Studies on aflatoxin detoxification in shelled corn by ensiling. J. Agr. Food Chem. 18(4), pp. 640-643.
- Mehrotra, S., and Srivastava, O. P. (Central Drug Research Institute, Lucknow, India). 1969. A new strain of Streptosporangium roseum Couch. J. Indian Bot. Soc. 48(3-4), pp. 261-263.*
- Muller, R. D., Carlson, C. W., Semeniuk, G., and Harshfield, G. S. (South Dakota State University, Brookings, South Dakota). 1970. The response of chicks, ducklings, goslings, pheasants, and poults to graded levels of aflatoxins. Poultry Sci. 49(5), pp. 1346-1350.
- Newton, J. W. 1970. Metabolism of D-alanine in Rhodospirillum rubrum and its bacilliform mutants. Nature (London) 228(5276), pp. 1100-1101.
- Scott, P. M.,¹ Walbeek, W. van,¹ Harwig, J.,¹ and Fennell, D. I. (¹Research Laboratories, Food and Drug Directorate, Ottawa, Ontario, Canada). 1970. Occurrence of a mycotoxin, ochratoxin A, in wheat and isolation of ochratoxin A and citrinin producing strains of Penicillium viridicatum. Can. J. Plant Sci. 50(5), pp. 583-585.

*Research supported by PL 480 funds.

Semeniuk, G.,¹ Harshfield, G. S.,¹ Carlson, C. W.,¹ Hesselstine, C. W., and Kwolek, W. F.² (¹South Dakota State University, Brookings, South Dakota; ²USDA Biometrical Services, Peoria, Illinois). 1970. Occurrence of mycotoxins in Aspergillus. In M. Herzberg, ed., Proc. First U.S.-Japan Conf. on Toxic Microorganisms, Honolulu, Hawaii, October 7-10, 1968, pp. 185-190. Unnumb. Pub., U.S. Dept. of Interior and UJNR Panels on Toxic Microorganisms. Washington, D.C.

Semeniuk, G.,¹ Harshfield, G. S.,¹ Carlson, C. W.,¹ Hesselstine, C. W., and Kwolek, W. F.² (¹South Dakota State University, Brookings, South Dakota; ²USDA Biometrical Services, Peoria, Illinois). 1971. Mycotoxins in Aspergillus. Mycopathol. Mycol. Appl. 43(2), pp. 137-152.

Shotwell, O. L., Hesselstine, C. W., Goulden, M. L., and Vandegrift, E. E. 1970. Survey of corn for aflatoxin, zearalenone, and ochratoxin. Cereal Chem. 47(6), pp. 700-707.

Stubblefield, R. D., Shannon, G. M., and Shotwell, O. L. 1970. Aflatoxins M₁ and M₂: Preparation and purification. J. Amer. Oil Chem. Soc. 47(10), pp. 389-390.

Taylor, N. W., and Orton, W. L. 1970. Association constant of the sex-specific agglutinin in the yeast, Hansenula wingei. Biochemistry 9(14), pp. 2931-2934.

Taylor, N. W., and Orton, W. L. 1971. Cooperation among the active binding sites in the sex-specific agglutinin from the yeast, Hansenula wingei. Biochemistry 10(11), pp. 2043-2049.

Vesonder, R. F., Stodola, F. H., Rohwedder, W. K., and Scott, D. B.¹ (¹Council for Scientific and Industrial Research, Pretoria, South Africa). 1970. 2-D-Hydroxyhexadecanoic acid: A metabolic product of the yeast Hansenula sydowiorum. Can. J. Chem. 48(13), pp.1985-1986.

2. Microbiology of Cereal Flours

Hesselstine, C. W., Haynes, W. C., Wickerham, L. J., and Ellis, J. J. 1970. History, policy, and significance of the ARS Culture Collection. In "Culture Collections of Microorganisms," eds. H. Iizuka and T. Hasegawa, Proc. Int. Conf. on Culture Collections, Tokyo, Japan, October 7-11, 1968, pp. 21-38. Tokyo.

Kurtzman, C. P., Rogers, R., and Hesselstine, C. W. 1971. Microbiological spoilage of mayonnaise and salad dressings. Appl. Microbiol. 21(5), pp. 870-874.

Kwon-Chung, K. J.,¹ and Fennell, D. I. (¹U.S. Dept. Health, Education and Welfare, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland). 1971. A new pathogenic species of Aspergillus. *Mycologia* 63(3), pp. 478-489.

Pridham, T. G. 1970. New names and new combinations in the order Actinomycetales. U.S. Dept. Agr., Tech. Bull. 1424, 55 pp.

Pridham, T. G. 1971. Nomenclature of bacteria with special reference to the order Actinomycetales. *Int. J. Syst. Bacteriol.* 21(2), pp. 197-206.

Wickerham, L. J., Kurtzman, C. P., and Herman, A. I. 1970. Sexuality in Candida lipolytica. In "Recent Trends in Yeast Research," ed. D. G. Ahearn, chap. 6, pp. 81-92. Atlanta.

Wickerham, L. J., and Page, F. C.¹ (¹University of Wisconsin, Rock County Campus, Janesville, Wisconsin). 1970. Cultivation and lyophilization of *Mastigina* sp. *J. Protozool.* 17(4), pp. 518-520.

D. NUTRIENT COMPOSITION

1. Nutrient Values of Cereal Grains

Grams, G. W., Blessin, C. W., and Inglett, G. E. 1970. Distribution of tocopherols within the corn kernel. *J. Amer. Oil Chem. Soc.* 47(9), pp. 337-339.

Krull, L. H., Gibbs, D. E., and Friedman, M. 1971. 2-Vinylquinoline, a reagent to determine protein sulphydryl groups spectrophotometrically. *Anal. Biochem.* 40(1), pp. 80-85.

E. ALLEVIATION OF WATER POLLUTION

1. Fermentation of Cereal Grain Processing Wastes

None.

F. MISCELLANEOUS

1. Japanese Beetle Control

Bulla, L. A., Jr. 1970. SEM of microorganisms. *Micro-Vues* 2(2), pp. 1-3.

- Bulla, L. A., Jr., Bennett, G. A., and Shotwell, O. L. 1970. Physiology of sporeforming bacteria associated with insects. II. Lipids of vegetative cells. J. Bacteriol. 104(3), pp. 1246-1253.
- Coulter, W. H., and Costilow, Ralph N. (Michigan State University, East Lansing, Michigan). 1970. The role of barbituric acid in the nutrition of Bacillus popilliae. Can. J. Microbiol. 16(9), pp. 801-807.
- Dulmage, H. T.,¹ and Rhodes, R. A. (¹USDA Entomology Research Division, Brownsville, Texas). 1971. Production of pathogens in artificial media. In "Microbial Control of Insects and Mites," eds. H. D. Burges and N. W. Hussey, chap. 24, pp. 507-540. London.
- Lingg, A. J., and McMahon, K. J. (Kansas State University, Manhattan, Kansas). 1969. Survival of lyophilized Bacillus popilliae in soil. Appl. Microbiol. 17(5), pp. 718-720.
- St. Julian, G., Bulla, L. A., Jr., and Hesseltine, C. W. 1971. Germination and outgrowth of Bacillus thuringiensis and Bacillus alvei spores viewed by scanning electron and phase-contrast microscopy. Can. J. Microbiol. 17(3), pp. 373-375.

WHEAT UTILIZATION (NORTHERN REGION)

Problems and Objectives

Production capability in excess of U.S. and foreign demand continues to be the dominant factor in the wheat economy of the U.S. Wheat research attempts to solve the problems associated with the development of markets for the full productive capacity of U.S. agriculture. The Northern Division research program seeks to improve yield and quality of wheat flour for use in foods and to find new industrial markets for wheat, particularly in the Pacific Northwest where corn is not grown.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Improve yield and quality of wheat milling fractions.
2. Convert wheat flour and starch to chemical products whose use will be economically advantageous to selected segments of the paper industry.

Research on industrial utilization of wheat starch and flour relates closely to and is integrated with similar studies on corn starch and flour. Results of such research are reported under appropriate subdivisions of "Corn Utilization."

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Milling and Fractionation

Purpose: The process of milling wheat has changed very little since the turn of the century. New approaches to milling and processing could improve economics or extend food utilization by providing wheat fractions with higher quality or specialized properties for specific food applications. Basic to research on milling and fractionation are studies on the microstructure of the wheat kernel and on the chemical and physical properties of wheat proteins, which are responsible for the unique ability of wheat flour to form elastic doughs. Research on wheat at the Northern Division is coordinated with research on food utilization of wheat conducted at the Western Division.

Progress: Analysis of representative hard red winter (HRW) wheat and soft red winter (SRW) wheat flour fractions from the break and reduction sections of a laboratory flour mill and of fine and coarse fractions produced by air classification of the resultant patent flours showed that the highest lysine values, expressed as a percentage of the protein, were found in reduction flour and coarse, air-classified residue. Antistatic agent added at the 2% level to SRW wheat and HRW wheat flours made them more free flowing and increased the response of SRW wheat flour to protein shifting during air classification by about 10%, but no increased response was found for the HRW wheat flour. Maximum moisture level to which HRW and SRW wheat flours could be conditioned with buffer solution or water in industrial type equipment was 27% before the flour particles became sticky. Fractions containing 20-22% (d.b.) protein were obtained in yields of 33-43% when dried cheese wheys of 14.5-15.8% protein content, 70-72% lactose, and high or intermediate level of lactose crystallinity were finally ground at moderate intensity and then air classified. As protein content increased, lactose content decreased over a range of 78-57% in the product fractions.

New methods have been successfully employed for examining differences in wheat proteins related to genetic variations governing wheat quality. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate separated subunits of reduced glutenin and gliadin and established number weight of subunits. Glutenin subunits were preparatively isolated by a combination of gel filtration and ion-exchange chromatography. The longer mixing time of the semi-dwarf Red River 68 flour was related to the composition and properties of its glutenin protein component.

The relationship of structure of wheat gluten proteins to functional properties was further investigated. Amino acid compositions of isolated peptides from protease digests establish that glutamine residues occur in sequence and gliadins differ from glutenin in polypeptide structure. Methods have been developed to establish location of disulfide bonds in gluten protein chains and to determine peptides in column effluents.

Examinations of flour doughs showed that hard wheats had large pockets of protein, whereas in soft wheats the protein was more evenly distributed. Numerous lipoprotein inclusions of various shapes and sizes were more numerous in the hard wheats than in the soft wheats. There were no other distinguishing features among the samples examined.

Dispersed gluten on a water surface formed angular beaded strands approximately 100 angstroms in diameter. In addition, doughnut-shaped particles approximately 200 angstroms in diameter with a

center hole approximately 67 angstroms in diameter could be seen. Both hard and soft wheat glutens formed these particles. Purified gliadin also showed some doughnut-shaped particles approximately 150 angstroms in diameter which is about half the size reported by shadowed or negative stain techniques.

Protein from the precursors of wheat Triticum monococcum(einkorn), Aegilops speltoides, and A. squarrosa was examined with the electron microscope. These species exhibit the viscoelastic properties of wheat protein; however, they have only one set of chromosomes (wheat has all three sets). Protein surface dispersion exhibited a weak protein structure similar to soft wheat. Triticale, on the other hand, exhibited a protein structure similar to hard wheat. Differences in the macromolecular structure of protein from wheat varieties and related species have eluded ordinary electron microscopic observations.

Molecular weight studies on gamma-1 gliadin, gamma-3 gliadin, gliadin with the high molecular weight component removed, and whole gliadin were carried out in a number of solvents by ultracentrifugation at sedimentation equilibrium. The gamma-1 gliadin and gamma-3 gliadin gave minimum molecular weights of 30,000 and 35,000, respectively, in 3 M urea plus 0.15 M KCl at pH 3.1. The molecular weights of gliadin with the high molecular weight component removed and of whole gliadin in 3 M urea plus 0.15 M KCl at pH 3.1 are 30,000 and 49,000, respectively.

Dye-binding studies with 0.8 and 1.0% wheat gluten in 0.002 N HCl indicated that two glutens from wheat with good breadbaking properties had lower absorbance at 650 and 700 nm than two glutens from wheat with poor breadbaking properties. Different spectra of gluten plus dye in aluminum lactate buffer vs. dye in aluminum lactate buffer showed that two good breadbaking glutens had higher absorbance at 500 nm than two poor breadbaking glutens.

Research studies under a PL 480 grant to the University of Poznan, Poznan, Poland, revealed that the lysine distribution was highest in the aleurone layer but a positive reaction was obtained in the germ and also in the starchy endosperm of wheat. A number of histochemical methods for tracing the distribution in wheat of tryptophan, tyrosine, and sulfhydryl and amino groups were investigated. Although in general the localization of staining reactions was the same for hard and soft wheats, staining was more intense in the subaleurone of hard wheats than in other parts of the starchy endosperm. In soft wheats the staining reaction was uniform throughout the starchy endosperm.

Publications and Patents

A. NEW AND IMPROVED FOOD PRODUCTS

1. Milling and Fractionation

Bietz, J. A., Huebner, F. R., and Rothfus, J. A. 1970. Chromatographic comparisons of peptic digests of individual gliadin proteins. Cereal Chem. 47(4), pp. 393-404.

Bietz, J. A., and Rothfus, J. A. 1970. Comparison of peptides from wheat gliadin and glutenin. Cereal Chem. 47(4), pp. 381-392.

Rothfus, J. A. 1970. Newer techniques in protein isolation and characterization. J. Amer. Oil Chem. Soc. 47(9), pp. 316-325.

Pfeifer, V. F., and Anderson, R. A. July 28, 1970. Reduction of strontium-90 content of intact cereal grains. U.S. Patent 3,522,056.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Acid-Modified Flour for Surface Sizing Paper

Aranyi, C.,¹ Gutfreund, K.,¹ Hawrylewicz, E. J.,¹ and Wall, J. S. (¹IIT Research Institute, Chicago, Illinois). July 28, 1970. Gluten hydrolysate derivatives and compositions comprising the same. U.S. Patent 3,522,197.

Brekken, R. A.,¹ Lancaster, E. B., and Wheelock, T. D.¹ (¹Iowa State University, Ames, Iowa). 1970. Fluidization of flour in a stirred, aerated bed. Part I. General fluidization characteristics. Chem. Eng. Progr. Symp. Ser. 66(101), pp. 81-90.

Brekken, R. A.,¹ Lancaster, E. B., and Wheelock, T. D.¹ (¹Iowa State University, Ames, Iowa). 1970. Fluidization of flour in a stirred, aerated bed. Part II. Solids mixing and circulation. Chem. Eng. Progr. Symp. Ser. 66(105), pp. 277-284.

Cluskey, J. E., and Wu, Y. V. 1971. Optical rotatory dispersion, circular dichroism, and infrared studies of wheat gluten proteins in various solvents. Cereal Chem. 48(3), pp. 203-211.

Dimler, R. J. 1970. Industrial adoptions and accomplishments. Rpt. Sixth Nat. Conf. Wheat Util. Res., held at Oakland, Calif., November 5-7, 1969. West. Util. Res. Develop. Div., U.S. Agr. Res. Serv., ARS 74-54, pp. 1-7

- Egan, L. P., Squires, T. G., and Vercellotti, J. R. (University of Tennessee, Knoxville, Tennessee). 1970. Acetylated aldose chlorides by reaction of aldose peracetates with zinc chloride-thionyl chloride. Carbohydr. Res. 14(2), pp. 263-266.
- Eskins, K., Dintzis, F., and Friedman, M. 1971. Photopolymerization of methyl acrylate in dimethyl sulfoxide. J. Macromol. Sci.-Chem. A5(3), pp. 543-548.
- Eskins, K., and Friedman, M. 1970. Graft photopolymerization of styrene to wheat gluten protein in dimethyl sulfoxide. J. Macromol. Sci.-Chem. A4(4), pp. 947-956.
- Eskins, K., and Friedman, M. 1970. Photoaddition of dimethyl sulfoxide to polypeptides. Photochem. and Photobiol. 12(3), pp. 245-247.
- Freeman, R. S. III,¹ Tsao, G. T.,¹ and Lancaster, E. B. (¹Iowa State University, Ames, Iowa). 1971. Absorption of iodine vapor by starch granules suspended in potassium iodide solution. Staerke 23(1), pp. 3-7.
- Friedman, M., and Cavins, J. F. Sept. 29, 1970. S- β -(4-Pyridylethyl)-L-cysteine. U.S. Patent 3,531,490.
- Friedman, M., Krull, L. H., and Cavins, J. F. 1970. The chromatographic determination of cystine and cysteine residues in proteins as S- β -(4-pyridylethyl)cysteine. J. Biol. Chem. 245(15), pp. 3868-3871.
- Inglett, G. E. 1970. Prospects for nonfood uses of gluten. Rpt. Sixth Nat. Conf. Wheat Util. Res., held at Oakland, Calif., November 5-7, 1969. West. Util. Res. Develop. Div., U.S. Agr. Res. Serv., ARS 74-54, pp. 144-155.
- Kaiser, E. T. (University of Chicago, Chicago, Illinois). 1970. Enzymatic and nonenzymatic reactions of cyclic sulfonate and sulfate esters. Accounts Chem. Res. 3, pp. 145-151.
- Li, K.-j.,¹ Wheelock, T. D.,¹ and Lancaster, E. B. (¹Iowa State University, Ames, Iowa). 1971. Thermal properties of soft white winter wheat flour. Chem. Eng. Progr. Symp. Ser. 67(108), pp. 17-23.
- Miller, D. L. 1970. A matter of economics. Wheat West, pp. 8-9 (January)
- Miller, D. L. 1970. Industrial alcohol from wheat. Rpt. Sixth Nat. Conf. Wheat Util. Res., held at Oakland, Calif., November 5-7, 1969. West. Util. Res. Develop. Div., U.S. Agr. Res. Serv., ARS 74-54, pp. 20-33.

Miller, D. L. 1970. Pulp and paper from wheat straw. Rpt. Sixth Nat. Conf. Wheat Util. Res., held at Oakland, Calif., November 5-7, 1969. West. Util. Res. Develop. Div., U.S. Agr. Res. Serv., ARS 74-54, pp. 34-38.

Rankin, J. C., Webber, R. A.,¹ and Robbins, P. J.¹ (¹Brown Company, Berlin, New Hampshire). 1970. On-machine surface sizing trials with acid-modified wheat flour. Tappi 53(8), pp. 1482-1485.

Tomalin, G., Trifunac, M., and Kaiser, E. T. (University of Chicago, Chicago, Illinois). 1969. The reaction of 4-nitrocatechol cyclic sulfate with α -chymotrypsin. J. Amer. Chem. Soc. 91 (3), pp. 722-726.

GRAIN SORGHUM UTILIZATION

Problems and Objectives

The growing importance of grain sorghum as a cash crop and the increasing magnitude of production challenge technology to establish a pattern of utilization that will maintain or increase the economic value of the crop. Because the bulk of the U.S. crop is grown in a relatively limited region west of the Mississippi River, there are geographic areas where freight transportation advantages should permit increased food and industrial usage of grain sorghum without significant displacement of other cereal grains. Food usage, now taking only about 1 percent of production, could be increased by improving quality, acceptability, and suitability of grain sorghum products for food. Limited industrial outlets for grain sorghum already exist. Processing techniques specifically adapted to grain sorghum should result in improved products that can maintain present industrial outlets against competition from synthetics as well as lead to increased industrial utilization.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Get needed data on composition and processing treatments for use in making new food products from grain sorghum.
2. Convert grain sorghum to new or improved industrial products via technology suited to the specific characteristics of this grain.

Some of the research on grain sorghum is closely related to similar studies on corn and wheat. Results of such research are reported under appropriate subdivisions of Corn Utilization and Wheat Utilization.

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Grain Sorghum Endosperm Products for Food Applications

Purpose: Although sorghum generally costs about 10% less than corn, only about 6% of the sorghum grain produced is used in U.S. food in contrast to 9% of the corn production. Over the next 10 years, population increases will generate increased demand for wheat-based food products in the principal grain-sorghum producing areas of the U.S. This increased demand could be supplied by supplementing wheat now required for these products with grain sorghum. To increase utilization of grain sorghum in food, compositional and genetic studies, new or improved methods of processing grain sorghum, and preparation of new or improved sorghum food products are needed.

Progress: A survey of different types of grain sorghum by optical microscopy showed only minor variations in structure. Thickness and starch content of pericarp was the most distinguishing characteristic. Hand-separated pericarp from different varieties accounted for 5.2 to 8.2% of the kernel. Pericarp starch granules appeared to be within the range of one to six microns in all varieties. Endosperm protein was found to be mostly in the form of bodies approximately two microns in diameter. The volume of these bodies is eight times that in corn. Matrix protein appears to be very limited in most areas. Examination of sorghum lines varying in protein, lysine, and digestibility showed no obvious difference in kernel structure. Scanning electron micrographs of the fractured surface of endosperm tissue showed that the separation occurred mostly through the cell contents and occasionally along cell walls. The cell walls were smooth with no adhering starch; only small bits of matrix protein were attached to the wall.

From the prolamine of grain sorghum flour, a fraction soluble in 95% ethanol at 9°C. was separated. In addition, the fastest migrating gel electrophoretic component of the prolamine was isolated by a combination of solubility difference and column chromatography.

The 95% ethanol-soluble fraction and the whole prolamine have identical gel electrophoretic patterns and similar amino acid composition, but the single component has several significant differences in amino acid content. For those proteins soluble in 95% ethanol at 9°C., the weight average molecular weight in 6 M guanidine hydrochloride at sedimentation equilibrium will depend not only upon protein concentration but also upon the pH of the solvent system. The molecular weight values in 6 M guanidine hydrochloride of the fastest electrophoretic component show a dependence upon initial protein concentration but no dependence upon the pH of the medium.

A series of wet-milling experiments was conducted to determine the effect of alkali-peeling on milling of grain sorghum. Grain was subjected to alkali-peeling at 160° F. for 2, 4, and 6 minutes. Alkali-peeling of the sorghum resulted in recovery of less (but whiter) starch and more germ of higher oil content, than is usually obtained from the whole grain. From an overall processing standpoint, the best time of peeling would be about 6 minutes.

Under grant research at Kansas State University, Manhattan, mild acid treatment of the reduced trisaccharide fraction from either glucoamylase I or II from Aspergillus niger caused a partial hydrolysis and formation of mono- and disaccharides. Lability of this glycoside bond to acid indicates the presence of a furanoside linkage in the heterogeneous trisaccharide units. Serine and threonine losses during alkaline borohydride treatment indicate the average carbohydrate moiety to be a disaccharide unit.

Publications

A. NEW AND IMPROVED FOOD PRODUCTS

1. Grain Sorghum Endosperm Products for Food Applications

- Anderson, R. A. 1971. An integrated process for dry-milling grain sorghum. Proc. 7th Biennial Grain Sorghum Res. and Util. Conf., Lubbock, Texas, March 2-4, 1971, pp. 65-69.
- Anderson, R. A. 1971. Dry-milling process for grain sorghum. Sorghum Newsletter 14, pp. 37-38.
- Blessin, C. W. 1971. Processing sorghum grain to improve nutritional quality and industrial uses. Proc. 7th Biennial Grain Sorghum Res. and Util. Conf., Lubbock, Texas, March 2-4, 1971, pp. 70-73.
- Cavins, J. F., Blessin, C. W., and Inglett, G. E. 1971. Amino acid infusion of grain sorghum. Sorghum Newsletter 14, p. 36.
- Deyoe, C. W., Shoup, F. K., Miller, G. D., Bathurst, J., Liang, D., Sanford, P. E., and Murphy, L. S. (Kansas State University, Manhattan, Kansas). 1970. Amino acid composition and energy value of immature sorghum grain. Cereal Chem. 47(4), pp. 363-368.

SOYBEAN UTILIZATION

Problems and Objectives

The pressing need for dietary protein and for food fat throughout the world poses a problem that urgently demands solution. U.S. soybeans could play a dominant role in alleviating these shortages in developing countries and elsewhere around the world, if soybean protein products can be successfully used in food products tailored to meet the various nutritional and palatability requirements and if opportunities for foreign utilization of soybean oil can be increased by development of inexpensive processes for insuring flavor stability. Solution of these problems would also contribute to increased domestic use of soybean food products and soybean oil. Nonfood usage of soybean oil has rather consistently accounted for about 10% of domestic disappearance. In view of the increasing production of soybeans and soybean oil, maintenance of nonfood markets requires continuing effort to develop products that can compete with synthetics derived from nonagricultural sources.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Producing high-protein foods based on full-fat soybean flour.
2. Removing objectionable flavors and other factors from soy products.
3. Increasing flavor stability of soybean oil.
4. Improving refining processes for soybean oil by reducing cost and associated water pollution.
5. Converting soybean oil to competitive industrial chemical products.

Progress Report

A. NEW AND IMPROVED FOOD PRODUCTS

1. Development of Processes for Improving Flavor and Acceptability of Edible Soybean Products

Purpose: Soybean products have found only limited outlets in foods in the U.S. despite their content of high-quality protein. The flavor of soy products is frequently unacceptable to consumers. The tendency to produce flatulence in some persons and the possible presence of certain antinutritional factors are also disadvantages.

Because of the potential importance of soybean products in improving the adequacy of diets in many segments of the U.S. population, processes are urgently needed for producing acceptable products that contain no antinutritional or other undesirable factors and that retain the functional properties that are critical to use of these products in many applications.

Progress: Extraction of defatted soybean flakes with azeotropic mixtures of hexane:ethanol or hexane:propanol-2 removed flavor components with minimal effects on protein solubility. Hexane:methanol also removed flavor components from defatted flakes, but extensive protein denaturation occurred with this solvent, and low yields of isolates were obtained from the extracted flakes. Flavor components and oil can be extracted from full-fat soybean flakes in a single step with hexane:ethanol, but solvent recovery problems were encountered because of solvent composition changes and phase separations. Shifts in apparent isoelectric points of soybean whey occur in the presence of phytate ion as determined by isoelectric focusing studies. Soybean saponins can be separated into 12 fractions by ion-exchange chromatography, and a new aglycone has been isolated.

Quantitative analyses of two bitter components, ethyl- α -D-galactoside and tryptophan, were completed. Results indicate that the amounts present in the flavor extracts are much below their flavor threshold level. Studies on lipoxygenase activity and flavor intensity values for beaniness and bitterness were initiated on developing soybeans at 15 intervals from 24 days after flowering to maturity. Lipoxygenase activity, measured as oxygen uptake, varied as much as fourfold throughout the growing period. Intensity of beany flavor was high and remained high throughout maturing period, whereas bitter intensity was low but increased during maturation. Taste panel evaluations showed no correlation of flavor with possible lipid oxidation as measured by lipoxygenase activity or thiobarbituric acid assay. An extremely bitter fraction with a threshold taste level of 0.02% has been prepared from full-fat flakes. The fraction still contains some impurities, but it is free of the other flavors associated with the original extract.

In connection with research on characterization of soybean lipoxygenase, a procedure for lipoxygenase oxidation of linoleic acid has been developed whereby hydroxy conjugated octadecadienoic acid (HCD) can be produced for laboratory experiments. Further scale-up to the 300-gram sample size has been attempted with crude soybean soaps. The oxidations were moderately successful with 40 to 60% of the available linoleic acid being converted to HCD. A preliminary cost study was made for the lipoxygenase oxidation of soybean soaps based on present data. The estimated total cost to produce HCD is 60.6 cents/pound for 100% HCD.

α -Galactosidase from a commercial source has been purified by a combination of ammonium sulphate fractionation and column chromatography. Its properties have been studied, and it has been successfully immobilized on cellulose and porous glass. On porous glass the enzyme shows very good stability. A mutant strain of NRRL 4869 has been used to make an α -galactosidase of high specific activity. Galactose dehydrogenase from Pseudomonas fluorescens has been immobilized on porous glass and shows fair stability with time as well as high activity against galactose.

At the University of Illinois, detailed analyses of the microflora in fecal matter of five human adult subjects fed a nonflatulent soy protein isolate diet and a flatulent defatted soy grit diet have been made. As in dogs, there are marked changes in the fecal microbial profile caused by these diets and the gas-producing ability of bacteria isolated from fecal matter derived from a flatulent diet changes greatly. Raffinose, when added to a soy protein isolate diet at a level equal to the total amount of raffinose plus stachyose in a flatulent soy grit diet, increased flatus production to a value nearly comparable to that produced with soy grits. Sucrose does not cause flatulence in humans fed protein isolates (see page 12).

In grant research at the University of Minnesota, 15 varieties of soybeans previously evaluated were planted, harvested, and reevaluated for protein efficiency ratio (PER), antitryptic and hemagglutinating activities. No significant differences were found between the two crops. Storage of soybeans at -10° and 30° for 30 months likewise had no effect on trypsin inhibitor or hemagglutinating activities. Previous work failed to show a correlation between content of sulfur-containing amino acids and PER when meals were fed raw. Five samples containing a wide range of sulfur amino acid contents were therefore heated and reevaluated for PER. Although heating increased PER of all samples, no correlation was found between sulfur content and PER.

At the Baylor College of Medicine, continued studies on young dogs maintained on a raw soybean diet showed that the concentrations of proteolytic enzymes in the pancreas decreased initially, but that adaptation occurred within 3 weeks of feedings and enzymatic activities returned to normal levels based on comparisons with dogs fed a casein control diet. Infusion of purified soybean trypsin inhibitor had no effect on the pancreas. These studies on young dogs together with previously reported chronic studies on adult dogs indicate that raw soybean meal has little effect on the pancreas with respect to size or to protein content and synthesis of enzymes.

Interaction of soybean hemagglutinin with animal cells has been studied under a PL 480 grant to the Weizman Institute of Science,

Rehovoth, Israel. In addition to its interaction with erythrocytes, soybean hemagglutinin agglutinates various somatic cell lines from tissue cultures which were transformed by viruses from normal to malignant ones. Agglutination reactions were specifically inhibited by low concentrations (0.05-0.1 μ moles/ml) of N-acetyl-D-galactosamine. Apparently an N-acetyl-D-galactosamine-like moiety is part of the receptor sites of the cell surface membranes. Soybean hemagglutinin can therefore be used for the detection of N-acetyl-D-galactosamine-like residues in cell membranes. Further fractionation studies indicate that the hemagglutinins are the only glycoproteins present in soybean whey. Two additional biologically active proteins, aspartate aminotransferase and malic dehydrogenase, have been isolated from defatted soybean meal.

In a completed study at the University of Tokyo, a standardized procedure for making kori-tofu (dried tofu) was developed. Salient features include rapidly chilling the fresh tofu to -10° C. in 1 to 2 hours and holding at -1 to -3° C. for 13 weeks. From 100 parts of soybeans, about 200 parts of fresh tofu and 80 parts of kori-tofu are obtained. Hawkeye was judged to be the best variety for kori-tofu; it rated high in yield, color, and brightness. Basic studies suggest that the ratio of 7S/11S proteins is important for kori-tofu. Browning is caused by a reaction between protein and oxidative products from unsaturated fatty acid in high humidities. Low storage temperature and humidities are desirable in protecting the dried tofu from browning.

2. Selective Hydrogenation of Soybean Oil

Purpose: Flavor instability of soybean oil has been attributed to the presence of linolenic acid among the component fatty acids of the oil. Linolenate can be removed by selective hydrogenation. However, when conventional nickel hydrogenation catalysts are used, excessive amounts of saturates and undesired trans isomers are formed. Research at the Northern Division is concerned with finding catalysts that will selectively remove linolenate while preserving the maximum polyunsaturation in the other fatty acids and minimizing the formation of saturates and trans isomers. Certain catalysts containing copper have been found to display a high degree of selectivity and are currently being intensively studied.

Progress: Copper-on-silica selective hydrogenation catalysts with high copper contents, obtained by new preparative methods, were more active and selective than Cu-Si catalysts with low copper loading when used at the same copper-oil ratio for partial hydrogenation of a soybean oil. Catalysts calcined at 350° C. were more active and selective than those calcined at 500° or 600° C. Addition of 0.001% reduced Ni (oil basis) to copper chromite catalyst allowed the reduction of the catalyst from 1% to 0.25% (oil basis). The reaction rate was nearly the same but there was some lowering of selectivity.

A laboratory-scale minaturized soybean oil processing plant (mini-refinery) has been assembled to study the feasibility of computer-controlled vegetable oil refining. The mini-refinery consists of four unit operations: alkali refining, bleaching, hydrogenating, and deodorizing. The development is now in the monitoring stage with two other stages to follow: (a) Monitoring with instructions to operator, and (b) "closing the loop" or having the computer directly control the process. Some transducers and specialized analytical processes and techniques are in use and others are in the planning stage. Incorporation of the recycle method for wash water to reduce pollution is planned.

Development and application of a room odor test as a means of evaluating cooking oils were undertaken. Tests were applied to various soybean-peanut oil mixtures, to copper-hydrogenated linseed oils, to all vegetable shortenings, to mixed fat shortenings, and to cooking oils modified by the addition of stabilizers, antifoam agents or chemical agents suspected of being fat oxidative deterioration products. Room odor tests have supplemented and given new information not obtainable with the autoxidative long-term storage evaluation tests. A newly devised ashing method called char-ashing permits the ashing of glyceride oils without flame burning. This technique allows the determination of a few parts per billion of iron and copper in oil with high accuracy by the atomic absorption technique.

In studies on the use of deuterium to elucidate the mechanism of hydrogenation, preliminary results indicate that copper catalysts induce greater isomerization and substitution of deuterium for hydrogen. The microreactor apparatus has been applied to simultaneous transesterification and isomerization of triglycerides with tetramethyl ammonium hydroxide. The microreactor procedure for location of double bonds has been improved by using solid triphenylphosphine to reduce ozonides. Gas chromatographic equivalent chain lengths have been determined for isomeric methyl octadecadienoates and trienoates.

The products of the reaction of fatty acids with deuterium and various catalysts have been examined by mass spectrometry to study exchange reactions so that they may be compared with bond migration and isomerization. By careful adjustment of the mass spectrometer, as little as .05 atoms of deuterium per molecule of methyl ester can be observed.

In addition to the "mini-refining" described above, several other aspects of the research have been significantly facilitated by application of computer guidance (see page 14).

Under contract research at the University of Minnesota, intermediates leading to 9 isomeric, carboxy labeled octadecenoic fatty acids (isomeric oleic acids) have been prepared, i.e., Δ -5, 6, 7, 8, 10, 11, 12, 13, and 14 octadecenoic acids. Synthesis of carboxy labeled Δ -2, 3, 16, and 17 octadecenoic acids have been attempted to complete the series of Δ 2 to 17 isomers. Work was begun to synthesize 18-hydroxystearic acid as a standard for omega-oxidation enzyme studies. The omega-oxidation of oleic acid by liver microsomes to 9-octadecene-1,18 dioic acid and 18-hydroxyoleic acid was carried out as the first enzymatic reaction.

Preparation of pure di- and triglycerides has continued at the University of Bombay, under a PL 480 grant, with a series of new glycerides prepared and in preparation. These glycerides contain saturated and trans monounsaturated fatty acids in the 1- and 3- positions and unsaturated fatty acids in the 2-position. Acids being used for introduction into the 1- and 3-positions are palmitic, stearic, and elaidic, and for the 2-position are oleic, linoleic, and linolenic. Information on the physical properties of some of these glycerides such as melting point, refractive index, density, and viscosity, is being obtained.

In other PL 480 studies at the Hebrew University of Jerusalem, a large quantity of highly purified campesterol has been prepared from soybean oil in order to confirm previous assumptions that campesterol is degraded by insects to cholesterol. A series of 7-dehydrosterols (campesterol and sitosterol), which are provitamin D, and fluoro-sterols derived from soybean sterols, are being tested for their biological availability by insects. The southern cowpea weevil has been added as another test insect to study cholesterol antagonists. A one-step photochemical bromination-debromination process has been discovered for the preparation of dehydrosterols in 65% yields. Preliminary tests have indicated that neither 7-dehydro- β -campesterol or 7-dehydro- β -sitosterol are being utilized by the larva of the hide beetle.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Nylon-Type Polyamides

Purpose: Nylon-9 has the highest melting point of nylons characterized by low moisture absorption. Of this group of nylons, nylon-11 and -12 are manufactured abroad and imported into the U.S. If available domestically at a competitive price, nylon-9 should replace these imported nylons and find ready market acceptance. Work at the Northern Division has shown that soybean oil is a preferred starting point for a synthetic route to nylon-9 that should be technically and economically feasible. The synthesis involves alcoholysis of the oil to alkyl soyate which is ozonized to a compound that is converted in one step to alkyl 9-aminononanoate. The Division's current program comprises process and product development of nylon-9.

Progress: Ammonium acetate was found to be a singularly effective catalyst for ammonolysis of vegetable oils. A copoly(ester-acetal) containing the pentaerythritol acetal of methyl azelaaldehyde as a comonomer formed a polar GLC column packing that was more heat-stable than conventional polyester phases. Work was initiated on preparation and purification of monomers from 9(10)-formylstearic acid obtained by the rhodium oxo process and on preparation of polymers from them.

At the Southern Research Institute, Birmingham, Alabama, under contract, progress in building up a supply of 9-aminononanoic acid monomer has been slow because of the low yields obtained in laboratory studies. Monomer synthesis is now being conducted in newly acquired pilot equipment, and faster progress is expected. The pilot synthesis has been carried out successfully through ozonolysis and esterification steps.

C. FOREIGN MARKET DEVELOPMENT

1. High-Protein Foods from Full-Fat Soybean Flours

Purpose: This research was originally undertaken as part of the Food for Peace Program under UNICEF and AID sponsorship to help alleviate the dietary deficiency of protein in developing countries. Work is now redirected towards the developing of cash markets in countries that are becoming increasingly able to pay dollars for U.S. soybeans. A new process is being developed for the manufacture of full-fat soybean flour based on high-temperature, short-time extrusion cooking. Solutions of problems involved in utilization of the full-fat flour, e.g. in beverages, are also being sought.

Progress: Preliminary investigations of the flatulence properties of our full-fat soy beverage powder, based on consumption level of three 8-ounce glasses of beverage per person per day suggest that flatulence may not be a serious problem for this product. Carbohydrate removal from soybeans to produce a nonflatulent base for soy beverages was studied based on the use of aqueous ethanol extraction. Fifty percent ethanol extraction gave the highest carbohydrate removal and good residual flavor but poor functionality for beverage production. Increased temperature improved flavor but decreased functionality.

Extruders for producing full-fat soy flour are now used commercially in Taiwan, Hong Kong, India, Venezuela, Mexico, South Africa, and Uganda. Under auspices of UNICEF, sets of machines for producing full-fat flour by the Village Process have been supplied since 1967 to Korea, Brazil, India, Philippines, Guatemala, and several countries in Africa. In December 1970, UNICEF sent 5 sets of machines to Tanzania.

D. ALLEVIATION OF WATER POLLUTION

1. Soybean Oil Refining Process

Purpose: In alkali-refining edible soybean oil, the crude oil is mixed with an aqueous solution of caustic soda and the aqueous phase containing phosphatides and fatty acid soaps is separated. The refined oil is then washed with water to further remove soap. This wash water which also contains emulsified oil is sent to municipal sewage disposal plants or discarded into rivers and streams where it contributes to water pollution. The refined and washed oil is dried and then heated with clay at an elevated temperature. The principal reason for this step, although it is termed "bleaching," is to further remove traces of soap which, if not removed, give a poor taste to the oil and also poison hydrogenation catalysts. The Northern Division has devised a process for washing alkali refined oil in which water is passed through the oil, then through ion-exchange resin and recycled. This process eliminates the problem of disposing of alkaline, soap-containing wash water, avoids loss of oil due to emulsions, and removes soap so effectively that the bleaching step can be omitted. Current research is directed towards developing the process to a point such that trials can be made in an operating soybean oil refining plant.

Progress: In pilot-plant tests, alkali refined soybean oil, washed by the continuous water recycle method and not bleached, could be hydrogenated as fast as or faster than bleached oil and significantly faster than oil washed conventionally with raw water. Recycle washed oil was lighter in color, more stable to oxidation, and had a lower content of trace metals than raw-water-washed oil. Nickel dissolved in hydrogenated soybean oil from nickel catalyst was 90% removed by recycle washing. Ethylene diamine tetraacetic acid added to recycle wash water increased the removal of copper and iron traces from alkali-refined soybean oil.

A contract has been awarded to Anderson-Clayton Foods, Dallas, Texas, for plant-scale investigation of the recycle washing process.

Publications and Patents

A. NEW AND IMPROVED FOOD PRODUCTS

1. Development of Processes for Improving Flavor and Acceptability of Edible Soybean Products

Arai, S., Noguchi, M., Kurosawa, S., Kato, H., and Fujimaki, M. (University of Tokyo, Tokyo, Japan). 1970. Applying proteolytic enzymes on soybean. 6. Deodorization effect of aspergillopeptidase A and debittering effect of Aspergillus acid carboxypeptidase. J. Food Sci. 35(4), pp. 392-395.*

Arai, S., Noguchi, M., Yamashita, M., Kato, H., and Fujimaki, M. (University of Tokyo, Tokyo, Japan). 1970. Applying proteolytic enzymes on soybean. Part VII. Properties of soy protein treated with microbial acid protease (molsin). Agr. Biol. Chem. 34(9), pp. 1338-1345.*

Arai, S., Noguchi, M., Yamashita, M., Kato, H., and Fujimaki, M. (University of Tokyo, Tokyo, Japan). 1970. Studies on flavor components in soybean. Part VI. Some evidence for occurrence of protein-flavor binding. Agr. Biol. Chem. 34(10), pp. 1569-1573.*

Arai, S., Yamashita, M., Kato, H., and Fujimaki, M. (University of Tokyo, Tokyo, Japan). 1970. Applying proteolytic enzymes on soybean. Part V. A nondialyzable bitter peptide in peptic hydrolyzate of soybean protein and its bitterness in relation to the chemical structure. Agr. Biol. Chem. 34(5), pp. 729-738.*

Cowan, J. C., Evans, C. D., Moser, H. A., List, G. R., Koritala, S., Moulton, K. J., and Dutton, H. J. 1970. Flavor evaluation of copper-hydrogenated soybean oils. J. Amer. Oil Chem. Soc. 47(12), pp. 470-474.

Dimler, R. J. 1970. Soy food opportunities through research. Soybean Dig. 31(1), pp. 17-23.

Dimler, R. J. 1970. New food products--soybeans. Proc. Conf. of Collaborators from Southern Agricultural Experiment Stations, New Orleans, Louisiana, March 16-17, 1970. U.S. Agr. Res. Serv., ARS 72-82, pp. 77-84.

Fujimaki, M., Kato, H., Arai, S., and Yamashita, M. (University of Tokyo, Tokyo, Japan). June 15, 1971. Enzymatic improvement of soybean flavor and stability. U.S. Patent 3,585,047.*

*Research supported by PL 480 funds.

- Fujimaki, M., Yamashita, M., Okazawa, Y., and Arai, S. (University of Tokyo, Tokyo, Japan). 1970. Applying proteolytic enzymes on soybean. 3. Diffusible bitter peptides and free amino acids in peptic hydrolyzate of soybean protein. J. Food Sci. 35(3), pp. 215-218.*
- Honig, D. H., Rackis, J. J., and Sessa, D. J. 1971. Isolation of ethyl α -D-galactopyranoside and pinitol from hexane-ethanol extracted soybean flakes. J. Agr. Food Chem. 19(3), pp. 543-546.
- Ikan, R., and Gottlieb, R. (Hebrew University, Jerusalem, Israel). 1970. Gas chromatography of tetra- and pentacyclic triterpenes. Isr. J. Chem. 8(4), pp. 685-689.*
- Ikan, R., Markus, A., and Bergmann, E. D. (Hebrew University, Jerusalem, Israel). 1970. A new synthesis of 7-dehydrosterols. Isr. J. Chem. 8(5), pp. 819-822.*
- Ikan, R., Markus, A., and Bergmann, E. D. (Hebrew University, Jerusalem, Israel). 1970. Synthesis of campesteryl acetate. ((24R)-24-methyl-3 β -acetoxysterol-5-ene) and its 24S-epimer. Steroids 16(5), pp. 517-522.*
- Ilany-Feigenbaum, J., Diamant, J., Laxer, Sh., and Pinsky, A. (Bar-Ilan University, Ramat-Gan, Israel). 1969. Japanese miso-type products prepared by using defatted soybean flakes and various carbohydrate-containing foods. Food Technol. 23(554), pp. 156-158.*
- Kawamura, S., Kasai, T., and Tanusi, S. (Kagawa University, Takamatsu, Japan). Feb. 9, 1971. Rapid microbiological production of alpha-galactosidase. U.S. Patent 3,562,113.*
- Kawamura, S., Tada, R., and Kasai, T. (Kagawa University, Takamatsu, Japan). 1970. Decomposition of soybean oil oligosaccharides by intestinal bacteria. IV. Formation of organic acids from the sugar mixture extracted from defatted soybean meal by a few strains of Escherichia coli. Tech. Bull. Fac. Agr. Kagawa Univ. 21(48), pp. 90-103.*
- Kawamura, S., Tanusi, S., and Tada, M. (Kagawa University, Takamatsu, Japan). 1970. Acid hydrolysis of soybean oligosaccharides at the isoelectric point of soybean globulins. Tech. Bull. Fac. Agr. Kagawa Univ. 21(48), pp. 84-89.*

*Research supported by PL 480 funds.

- Kawamura, S., Torigoe, H., and Kasai, T. (Kagawa University, Takamatsu, Japan). 1970. Decomposition of soybean oligo-saccharides by intestinal bacteria. V. α -Galactosidase activity of selected strains of Escherichia coli outside and inside the cell. Tech. Bull. Fac. Agr. Kagawa Univ. 21(48), pp. 104-109.*
- Komoda, M., and Harada, I. (Sugiyama Chemical Research Institute, Tokyo, Japan). 1970. Interaction of tocopherol with unsaturated fatty esters. J. Amer. Oil Chem. Soc. 47(7), pp. 249-253.*
- Lis, H., Sela, B.-A., Sacha, L., and Sharon, N. (The Weizmann Institute of Science, Rehovoth, Israel). 1970. Specific inhibition by N-acetyl-D-galactosamine of the interaction between soybean agglutinin and animal cell surfaces. Biochim. Biophys. Acta 221(3), pp. 582-585.*
- Meyerson, S.,¹ Fenselau, C.,² Young, J. L.,² Landis, W. R.,³ Selke, E., and Leitch, L. C.⁴ (¹American Oil Co., Whiting, Indiana; ²Johns Hopkins University, Baltimore, Maryland; ³National Institutes of Health, Bethesda, Maryland; ⁴National Research Council, Ottawa, Ontario, Canada). 1970. Reactions of aliphatic aldehydes under electron-impact. Org. Mass Spectrom. 3., pp. 689-707.
- Noguchi, M., Arai, S., Kato, H., and Fujimaki, M. (University of Tokyo, Tokyo, Japan). 1970. Applying proteolytic enzymes on soybean. 2. Effect of aspergillopeptidase A preparation on removal of flavor from soybean products. J. Food Sci. 35(3), pp. 211-214.*
- Rackis, J. J., Honig, D. H., Sessa, D. J., and Cavins, J. F. 1971. Soybean whey proteins--recovery and amino acid analysis. J. Food Sci. 36(1), pp. 10-13.
- Rackis, J. J., Honig, D. H., Sessa, D. J., and Steggerda, F. R.¹ (¹University of Illinois, Urbana, Illinois). 1970. Flavor and flatulence factors in soybean protein products. J. Agr. Food Chem. 18(6), pp. 977-982.
- Rackis, J. J., Sessa, D. J., Steggerda, F. R.,¹ Shimizu, T.,¹ Anderson, J., and Pearl, S. L.¹ (¹University of Illinois, Urbana, Illinois). 1970. Soybean factors relating to gas production by intestinal bacteria. J. Food Sci. 35(5), pp. 634-639.

*Research supported by PL 480 funds.

- Sela, B.-A., Lis, H., Sharon, N., and Sachs, L. (Weizmann Institute of Science, Rehovoth, Israel). 1970. Different locations of carbohydrate-containing sites in the surface membrane of normal and transformed mammalian cells. *J. Membrane Biol.* 3(3), pp. 267-279.*
- Selke, E., Moser, H. A., and Rohwedder, W. K. 1970. Tandem gas chromatography-mass spectrometry analysis of volatiles from soybean oil. *J. Amer. Oil Chem. Soc.* 47(10), pp. 393-397.
- Sessa, D. J., Abbey, K. J., and Rackis, J. J. 1971. Tryptophan in soybean meal and soybean whey proteins. *Cereal Chem.* 49(3), pp. 321-327.
- Spadaro, J. J.,¹ and Miller, D. L. (¹Southern Marketing and Nutrition Research Division, New Orleans, Louisiana). 1971. New products from oilseeds. *Proc. 19th Cottonseed Processing Clinic*, held at New Orleans, Louisiana, February 16-17, 1970. U.S. Agr. Res. Serv., ARS 72-84, pp. 57-61.
- Wang, L. C. 1971. Effect of phytate on isoelectric focusing of soybean whey proteins. *Cereal Chem.* 48(3), pp. 229-238.
- Wang, L. C. 1971. Isoelectric point differences in commercial soybean trypsin inhibitors. *Cereal Chem.* 48(3), pp. 303-312.
- Wolf, W. J. 1970. Soybean proteins: Their functional, chemical, and physical properties. *J. Agr. Food Chem.* 18(6), pp. 969-976.
- Wolf, W. J., and Thomas, B. W. 1971. Ion-exchange chromatography of soybean saponins. *J. Chromatogr.* 56(2), pp. 281-293.

2. Selective Hydrogenation of Soybean Oil

- Bitner, E. D., Lanser, A. C., and Dutton, H. J. 1970. MRA microreactions for lipid analysis. *Lipids* 5(8), pp. 707-712.
- Hites, R. A. 1970. Quantitative analysis of triglyceride mixtures by mass spectrometry. *Anal. Chem.* 42(14), pp. 1736-1740.
- Koritala, S. 1970. Selective hydrogenation with copper catalysts: III. Hydrogen addition and isomerization. *J. Amer. Oil Chem. Soc.* 47(8), pp. 269-272.
- Koritala, S. 1970. Hydrogenation of unsaturated fatty esters with copper-chromite catalyst: Kinetics, mechanism and isomerization. *J. Amer. Oil Chem. Soc.* 47(12), pp. 463-466.

* Research supported by PL 480 funds.

- Koritala, S., Butterfield, R. O., and Dutton, H. J. 1970. Selective hydrogenation with copper catalysts: II. Kinetics. J. Amer. Oil Chem. Soc. 47(8), pp. 266-268.
- Koritala, S., and Scholfield, C. R. 1970. Selective hydrogenation with copper catalysts: I. Isolation and identification of isomers formed during hydrogenation of linolenate. J. Amer. Oil Chem. Soc. 47(8), pp. 262-265.
- Koritala, S., and Selke, E. 1971. Selective hydrogenation with copper catalysts: IV. Reaction of stearolate, oleate and conjugated esters with deuterium. J. Amer. Oil Chem. Soc. 48(5), pp. 222-227.
- Mounts, T. L., Dutton, H. J., and Glover, D.¹ (¹Bradley University, Peoria, Illinois). 1970. Conjugation of polyunsaturated acids. Lipids 5(12), pp. 997-1005.
- Scholfield, C. R., and Dutton, H. J. 1971. Equivalent chain lengths of methyl octadecadienoates and octadecatrienoates. J. Amer. Oil Chem. Soc. 48(5), pp. 228-231.
- Scholfield, C. R., and Koritala, S. 1970. A simple method for preparation of methyl trans-10, cis-12 octadecadienoate. J. Amer. Oil Chem. Soc. 47(8), p. 303.
- Scholfield, C. R., Mounts, T. L., Butterfield, R. O., and Dutton, H. J. 1971. Competitive hydrogenation rates of isomeric methyl octadecenoates. J. Amer. Oil Chem. Soc. 48(5), pp. 237-239.

B. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Nylon-Type Polyamides

- Awl, R. A., Pryde, E. H., Weisleder, D., Rohwedder, W. K., and Cowan, J. C. 1971. Alkyl azelaaldehydates of high purity from alkyl soyates: Preparation and properties. J. Amer. Oil Chem. Soc. 48(5), pp. 233-236.
- Frankel, E. N. Nov. 24, 1970. cis-Retaining selective hydrogenation of vegetable oils. U.S. Patent 3,542,821.
- Frankel, E. N. 1971. Methyl 9(10)-formylstearate by selective hydroformylation of oleic oils. J. Amer. Oil Chem. Soc. 48(5), pp. 248-253.
- Frankel, E. N., Thomas, F. L., and Cowan, J. C. 1970. Stereo-selective hydrogenation of model compounds and preparation of tailor-made glycerides with chromium tricarbonyl complexes. J. Amer. Oil Chem. Soc. 47(12), pp. 497-500.

Kohlhase, W. L., Pryde, E. H., and Cowan, J. C. 1971. Catalytic effects in the ammonolysis of vegetable oils. J. Amer. Oil Chem. Soc. 48(6), pp. 265-270.

Pryde, E. H., and Cowan, J. C. 1971. Ozonolysis. In "Topics in Lipid Chemistry," vol. 2, ed. F. G. Gunstone, pp. 1-98. London.

2. Lubricants

Bailar, J. C., Jr., Itatani, H., Crespi, M. J., and Geldard, J. (University of Illinois, Urbana, Illinois). 1967. Some recent developments in coordination chemistry. Advan. Chem. Ser. No. 62, pp. 103-113.

Friedrich, J. P. 1970. Wheels make high-pressure research roll. Ann. N.Y. Acad. Sci. 172, Art. 9, pp. 155-174.

Friedrich, J. P. 1971. High-pressure R&D on wheels. Chem. Technol. 1(1), pp. 52-55.

C. FOREIGN MARKET DEVELOPMENT

1. High-Protein Foods from Full-Fat Soybean Flours

Bookwalter, G. N., Mustakas, G. C., Kwolek, W. F.,¹ McGhee, J. E., and Albrecht, W. J. (¹USDA Biometrical Services, Peoria, Illinois). 1971. Full-fat soy flour extrusion cooked: Properties and food uses. J. Food Sci. 36(1), pp. 5-9.

Dimler, R. J. 1970. The role of agriculture in meeting the protein gap. Part 2 (morning session, November 26), Seminar on Protein Food Promotion, held at The Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand, November 22-December 1.

Mustakas, G. C. 1971. Extrusion cooking, a new development for soy protein foods. Proc. Central States Section, American Association of Cereal Chemists, 12th Annual Symp. "Extrusion: Process and Product Development," held at St. Louis, Missouri, February 12-13.

Mustakas, G. C., Albrecht, W. J., Bookwalter, G. N., McGhee, J. E., Kwolek, W. F.,¹ and Griffin, E. L., Jr. (¹USDA Biometrical Services, Peoria, Illinois). 1970. Extruder-processing to improve nutritional quality, flavor, and keeping quality of full-fat flour. Food Technol. 24(11), pp. 1290-1296.

Mustakas, G. C., Albrecht, W. J., Bookwalter, G. N., Sohns, V. E., and Griffin, E. L., Jr. 1971. New Process for low-cost, high-protein beverage base. Food Technol. 25(5), pp. 534-538, 540.

D. ALLEVIATION OF WATER POLLUTION

1. Soybean Oil Refining Process

Black, L. T. 1970. Direct determination of sodium in soybean oil by flame photometry. J. Amer. Oil Chem. Soc. 47(9), pp. 313-315.

FLAX UTILIZATION

Problems and Objectives

Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. Recently, annual domestic use of linseed oil has declined to 240 million pounds from a postwar high of over 700 million pounds in 1950. This decrease came primarily by displacement by synthetic materials capable of better performance, particularly in coatings. To restore the level of use of linseed oil, new or expanded markets are urgently needed. The most promising route to achievement of this goal is development of improved protective coating products that can compete with synthetics. Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multi-billion-pound annual market for products of the organic chemical industry.

Major objectives of current research are to develop and evaluate alternative ways for:

1. Improving durability of linseed emulsion paints.
2. Establishing economically feasible methods of curing and/or protecting concrete by use of linseed oil.
3. Converting linseed oil by economical processes to products that can compete in the industrial chemical market.

Progress Report

A. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Curing and Protecting Concrete

Purpose: Research at the Northern Division has demonstrated that coating concrete with boiled linseed oil applied as a solution in mineral spirits effectively protected air-entrained concrete from freeze-thaw damage and the effects of de-icing chemicals. This treatment is now used by many States and toll authorities. Later a linseed oil emulsion was devised that could be used not only to protect concrete but also to cure freshly poured concrete. The emulsion is now produced commercially by four companies. The most recent development is that preliminary results of exploratory tests suggest that use of the emulsion to coat porous and alkali-reactive aggregates improves their performance as a component of Portland cement concrete. Present studies are directed towards learning more about how linseed oil functions in curing and protecting concrete; overcoming various problems encountered in use of

linseed oil emulsion to cure and protect concrete; obtaining additional performance data that will encourage additional States to adopt the emulsion; and exploring the value of linseed oil in upgrading unsound aggregate.

Progress: Contract work on coating poor aggregate with linseed oil emulsions at Kansas State University, Manhattan, was continued. Incidental problems regarding the best way to treat aggregate were worked out. Nominal coating rates of 0.04, 0.08, 0.12, and 0.16 pounds of oil per square yard appear suitable for initial studies. Water absorption studies showed that absorption of water into aggregate decreased from 2.5% (no treatment) to 1.2% (0.16 pounds per square yard). Laboratory tests on the effect of concrete curing agents containing linseed oil on curing efficiency showed that with proper formulation, no loss in strength or surface hardness occurred with the oil-treated samples compared to moist cures. Studies designed to relate penetration or time of application of linseed oil antispalling compounds to durability of concrete revealed that deepest penetration and best durability is achieved when the oil is applied after the concrete is 14 days old.

2. Improved Durability of Linseed Oil Emulsion Paints

Purpose: In recent years, the market for linseed oil in oil-based paints has greatly declined due to the popularity of synthetic latex paints for exterior use. Linseed emulsion paints were developed in response to this competition and were commercially available for several years. These paints were, however, not a commercial success because some of their properties, especially tint retention and durability during the first year, were inferior to those of the synthetic-latex paints. Lack of water permeability and flexibility are also important factors that reduce durability by leading to blistering and cracking. Research is needed to improve the properties of linseed emulsion paints and thereby enhance their competitive position. Opportunities also exist to capture a share of the market for water-based industrial coatings by developing new rapid-drying, hard, durable paint vehicles based on linseed oil.

Progress: Scanning electron micrographs of aged paint films substantiate the claim that both solvent- and water-based linseed oil paints are at least as good and often better than the best exterior latex paints commercially available. Outdoor exposure of a total of 17 paints representative of all three types revealed no cracking or blistering and only a moderate amount of mildew the degree of which was independent of type of vehicle in the paint. Chalking was rather heavy in all paint films. Adherence between vehicle and pigment and extender particles was better in linseed oil paints than in latex paints.

Thirty alkyd-type polyesteramides and urethane-polyesteramides have been prepared and their film properties evaluated in the laboratory. The

better films dry within 2 to 6 minutes to form hard, glossy, chemical resistant films with excellent adhesion to metal. Film properties can be varied with polymer composition. The simplicity of the preparative procedure should make these products attractive for commercial development. Polyacetals and poly(ester-acetals) from hydroformylated linseed oil have been prepared from polyols and dibasic acids. The products show promise as protective coatings.

Dark-colored polyesteramides result from the formation of a colored complex between N,N-bis(2-hydroxyethyl)linseed amide (HELA), Cu and oxygen. This color can be induced in purified HELA by addition of Cu and oxygen. Ethylenediamine tetracetic acid may be used to prevent color formation. A new preparative technique for polyesteramide (alkyd-type cook) may also offer a solution to the color problem.

Inspections with the scanning electron microscope of linseed oil films containing zinc oxide show that the pigment-vehicle interface influences both water sensitivity and film strength. Matching surface properties of pigments and linseed oil should lead to paints with improved film properties. An equation relating surface tension to solubility parameters appears useful for predicting effects of pigment surface alteration on phenomena such as spreading, wetting, and adhesion. It may be possible to use hydrogen bonding to increase or decrease wetting and adhesion in a paint system.

Application of tris(triphenylphosphine) chlororhodium catalysts for conjugation of soybean oil led to variable results. The best result corresponded to a 65% conversion of the available diene to conjugated diene. Dimsyl sodium (sodium salt of dimethyl sulfoxide) in dimethylsulfoxide (DMSO) converted methyl linoleate to 96% conjugated linoleate in 2 hr. at room temperatures. Conjugation of soybean oil has been achieved with dimsyl sodium in DMSO-tetrahydrofuran.

A new rhodium-triphenylphosphine hydroformylation catalyst was found which is more selective than the conventional cobalt carbonyl catalyst. Olive fatty esters and acids were converted into 9(10)-formyl derivatives in very good yields. Polyunsaturated oils gave a mixture of saturated and unsaturated products containing one or more formyl groups per fatty acid. When hydroformylation was interrupted, cis unsaturated aldehyde oils were obtained which were useful in coating studies. Formyl esters were catalytically autoxidized to the carboxy esters or hydrogenated to the hydroxymethyl esters. Various diesters of carboxystearic acid are being evaluated as lubricants. Various acetals and an enol ether derivative were made and showed promise as plasticizers.

Publications

A. NEW AND IMPROVED INDUSTRIAL PRODUCTS

1. Curing and Protecting Concrete

None.

2. Improved Durability of Linseed Oil Emulsion Paints

Cowan, J. C. 1971. Linseed oil for edible use: Preliminary studies. Proc. 40th Annual Flax Inst. of the U.S., Minneapolis, Minn., November 12-13, 1970, pp. 28-30.

DeJarlais, W. J., and Gast, L. E. 1971. Conjugation of polyunsaturated fats: Methyl linoleate with tris(triphenylphosphine) chlororhodium. J. Amer. Oil Chem. Soc. 48(1), pp. 21-24.

DeJarlais, W. J., and Gast, L. E. 1971. Conjugation of polyunsaturated fats: Activity of some group VIII metal compounds. J. Amer. Oil Chem. Soc. 48(4), pp. 157-159.

Eissler, R. L., and Baker, F. L. 1971. Critical pigment volume concentration in linseed oil films. Appl. Polym. Symp. No. 16, pp. 171-182.

Eissler, R. L., Zgol, R., and Stolp, J. A. 1970. Solubility parameters applied to sedimentation volumes of pigments. J. Paint Technol. 42(548), pp. 483-489.

Khoe, T. H., Gast, L. E., and Cowan, J. C. 1971. New methacrylate and polyacetal coatings from linseed oil. Proc. 40th Annual Flax Inst. of the U.S., Minneapolis, Minn., November 12-13, 1970, pp. 24-26.

McManis, G. E. 1970. Infrared absorption spectra of vinyl esters of carboxylic acids. Appl. Spectrosc. 24(5), pp. 495-498.

McManis, G. E., Swain, E. W., Gast, L. E., and Cowan, J. C. 1971. Color stability of films from conventional and emulsion paints containing linseed oil. Effects of methyl azelaaldehyde. J. Paint Technol. 43(554), pp. 53-58.

- Schneider, W. J., McManis, G. E., Swain, E. W., and Gast, L. E. 1970. Polyesteramides from linseed and soybean oils for protective coatings. Color stability of diisocyanate-modified polymers. J. Paint Technol. 42(548), pp. 493-497.
- Schwab, A. W., and Gast, L. E. 1970. Free radical addition of hydrogen sulfide to conjugated and nonconjugated methyl esters and to vegetable oils. J. Amer. Oil Chem. Soc. 47(10), pp. 371-373.

CRAMBE UTILIZATION

Problems and Objectives

Crambe, a new oilseed crop commercialized in 1965, is the first plant included in the research program on new crops to achieve this status. Crambe seed oil is rich in erucic acid. Several industrial uses already exist for erucic acid as well as for imported rapeseed oil, which formerly was the only source of this acid. However, to insure optimum development of crambe as a new commercial crop, possible markets for crambe oil and erucic acid must be explored and those with the greatest industrial potential must be identified and exploited. In addition, since economic value to the farmer and to industry of any oilseed crop is much greater if the meal left after extraction of the oil can be utilized as a palatable and nutritious feed for animals, suitable processes are required to realize fully the anticipated nutritional qualities and to insure maximum acceptability to different types of animals.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Developing new industrial chemical products from crambe oil.
2. Improving protein feed supplements from crambe.

Progress Report

A. NEW AND IMPROVED INDUSTRIAL AND FEED PRODUCTS

1. Industrial Nylons and Special-Purpose Plasticizers

Purpose: The high content (55-60%) of the 22-carbon monounsaturated acid, erucic acid, uniquely differentiates crambe oil from other domestic vegetable oils that contain 18-carbon acids almost exclusively. Chemical modification of erucic acid results in a variety of potentially useful products that cannot be conveniently obtained from any other starting material. Erucic acid derived from imported rapeseed oil is commercially available and enjoys a few well-established industrial uses. Domestically produced crambe oil should be a more economical source of erucic acid. However, to realize this potential, additional uses for crambe oil and erucic acid are needed. Present research at the Northern Division is concerned with nylons-13 and -1313, which are derived from brassylic acid obtained by ozonization of erucic acid, and with certain esters of brassylic and erucic acids as special-purpose plasticizers.

Progress: Nylon-13 and -1313: At Southern Research Institute, Birmingham, under contract, crude brassylic acid has been prepared at a rate of 11-12 pounds per working day by pilot-scale ozonolysis of erucic acid. Yields, generally near 70%, equal those obtained in laboratory-scale experiments. Refinements in techniques for handling and washing the crystalline product have resulted in improved yields and color purity of the crude brassylic acid relative to initial pilot-scale preparations. When purified by further recrystallization, the acid is essentially colorless with a melt color of less than 40 APHA units. Quantities of brassylic acid now on hand are nearly sufficient for further planned work. Pilot-scale production of the dinitrile is complete. Above melt temperature, brassylic acid is readily converted to the corresponding diamide and subsequently to the dinitrile in 89% yield by anhydrous ammonia with polyphosphoric acid catalyst. The crude dinitrile is better than 96% pure by GLC analysis. Equipment is being installed to handle subsequent steps in the nylon-1313 synthesis, reduction of the dinitrile to diamine and melt polymerization of tridecane-1,13-diammonium brassylate.

Research at the Northern Division has shown that formation of acetals and acids are principal side reactions that lower aldehyde yields in the reductive ozonolysis of methyl erucate in solutions containing alcohols. Pelargonic acid and brassylic acid half ester are not formed in significant amounts during ozonolysis. They are found after distillation of the products and appear to be derived from acetal precursors. Ozonization in acetic acid eliminates acetals, suppresses acid formation, and allows optimum yields of 84% nonanal and 80% methyl 12-formyldodecanoate from 97% conversion of methyl erucate. Prolonged exposure to ozone reduces yields of these aldehydes. Oxidative decomposition of the optimum ozonization product produces up to 86% yield of pelargonic acid and 83% of brassylic acid half ester. In general, yields of nonanal and pelargonic acid parallel those of methyl 12-formyldodecanoate and brassylic acid half ester, respectively, throughout the ozonization and oxidation reactions. Analysis of the 9-carbon products provides a convenient means of following ozonolysis.

Special-Purpose Plasticizers: More than 2,000 strips of internally and externally plasticized vinyl chloride films were tested for tensile strengths at yield and at break, percent elongation, total energy before break, and stress versus stretch relationships. Preliminary calculations showed that three copolymers of vinyl chloride (ones with 2-methyl-pentyl vinyl brassylate, nonyl vinyl brassylate, and vinyl stearate as the other comonomer) form good clear films. For better comparison of tensile performances, a new parameter was derived from each integrated peak area traced on the tensile strength/elongation graph. This parameter, called "Capacity for Tensile Work (CTW)" represents each film's inherent capacity to perform work. Unlike the earlier expressions of energy in inch-pounds or in centimeter-grams, CTW is expressed in ergs per cubic millimeter of plastic material.

Allyl esters of brassylic, azelaic, and azelao-brassylic acids were prepared in large quantities and each diester was purified by distillation. The amounts recovered were 9.4 kg., 1.3 kg., and 0.6 kg., respectively. Ionox 330, a high molecular weight aromatic inhibitor, was selected to prevent spontaneous free radical polymerization of the allyl monomers during distillation at elevated temperatures. Purity of the monomers was determined by infrared, ultraviolet, gas chromatographic, and thin-layer chromatographic methods. Diallyl brassylate was 96% pure, mixed with 4% of diester homologs; diallyl azelate was 90% pure; and the azelao-brassylate (or mixed crambe diesters) contained 36% azelaic and 47% brassylic diesters. Gel time and polymerization parameters were investigated. Preparation of large quantities of liquid prepolymers was started.

Under a PL 480 grant to the Institute of General Chemistry, Warsaw, Poland, samples of five triglycerides containing erucic acid have been prepared and sent to the Northern Division as follows: Trierucin (50 g.); 1-erucoyl-2,3-diolein (30 g.); 1-oleoyl-2,3-dierucin (30 g.); 2-erucoyl-1,3-diolein (50 g.); 2-oleoyl-1,3-dierucin (50 g.). The four diacid triglycerides were made by synthetic sequences designed to ensure positional specificity of acyl group attachment. For the asymmetric triglycerides the intermediate 1-monoglycerides were obtained by acylation of 1,2-isopropylidene glycerol with the proper fatty acyl chloride. Analogously, the 2-monoglyceride precursors of the two symmetric diacid triglycerides were prepared by acylation of 1,3-benzylidene glycerol. In both cases, boric acid was used to catalyze the subsequent removal of blocking groups, i.e., acetone and benzaldehyde respectively. Then conversion of monoglycerides to triglycerides was accomplished by mixing the former and the appropriate fatty acyl chloride in chloroform with pyridine added to trap the generated hydrochloric acid. Final products were purified by crystallization and column chromatography.

2. Protein Feed Supplements

Purpose: After extraction of the oil from crambe seed, the residue contains a large percentage of high-quality protein. However, like certain other oilseed meals, crambe meal contains antinutritional factors that restrict its utilization as an animal feed supplement and, therefore, limit its price value. As a result, expansion to maximum market potential is curtailed since the oil must sell for a higher price to compensate for restricted usage and lower value of the meal. Methods of processing crambe are sought that will remove or deactivate antinutritional factors in crambe meal and thereby provide a protein supplement having maximum nutritional quality and acceptability.

Progress: Pilot-plant studies indicate that prepress-solvent should be recommended as the process for use in proposed future contract studies. Studies on ferrous sulfate cooking of crambe meals to decompose thioglucosides have been completed. Rats fed these meals at a 30% diet level gained weight to the extent of 70% compared to the basal control. Some thyroid, liver, and kidney hypertrophy was found. In a present laboratory pilot-plant program, a vacuum belt filter has been installed and tested. This unit will be used to study complete removal of thioglucosides from defatted crambe meal by water extraction.

Surprisingly, the rate of epi-progoitrin (e-PG) extraction from intact dehulled crambe seed is nearly independent of the volume of water employed. Estimated cost per ton (based on crambe as harvested--i.e., with hull--rather than as extracted without the hull) for three extractions with only sufficient water to provide a mechanically manageable slurry is less than \$6 per ton. The e-PG can be recovered from the steep liquor by an ion exchange procedure. Very high levels of the extracted meals in the diets of rats produced an unexplained growth retardation not due to e-PG derived substances or decrease in available lysine. In view of the excellent PER values found for the extracted meal at lower dietary levels, this growth retardation is particularly anomalous and is under further study.

Fractionation by ammonium sulfate precipitation of crude soluble thioglucosidase from crambe seed prepared in the presence of ferrous ion gives two fractions differing in the aglucon products formed from e-PG and in response to ascorbate: (a) At pH 5.3, the fraction precipitating between 50 and 60% saturation produces primarily 1-cyano-2(S)-hydroxy-3-butene and 1-cyano-2(S)-hydroxy-3,4-epithiobutanes and is activated by ascorbate; (b) the fraction at 60-70% saturation gives cyanobutene and goitrin and is relatively indifferent to ascorbate addition. Epithiobutane production is extremely labile.

It was confirmed that the progoitrins in seeds of Selenia grandis, a crucifer plant, consist of partly racemic S-2-hydroxy-3-butenyl-glucosinolate. This observation demonstrates that epimeric mixtures may occasionally be formed in nature. The glucosinolate content of over 200 accessions of Brassica seed has been estimated as a first step in a breeding program to reduce the amounts of these compounds in oilseeds from the crucifers.

Publications

A. NEW AND IMPROVED INDUSTRIAL AND FEED PRODUCTS

1. Industrial Nylons and Special-Purpose Plasticizers

Carlson, K. D., Weisleder, D., and Daxenbichler, M. E. 1970. Intramolecular hydrogen bonding. An infrared and nuclear magnetic study of diastereomeric episulfides. J. Amer. Chem. Soc. 92(21), pp. 6232-6238.

Daxenbichler, M. E., VanEtten, C. H., Hallinan, E. A., Earle, F. R., and Barclay, A. S.¹ (¹USDA Crops Research Division, Beltsville, Maryland). 1971. Seeds as sources of L-dopa. J. Med. Chem. 14(5), pp. 463-465.

VanEtten, C. H., and Daxenbichler, M. E. 1971. Formation of organic nitriles from progoitrins in leaves of Crambe abyssinica and Brassica napus. J. Agr. Food Chem. 19(1), pp. 194-195.

2. Protein Feed Supplements

Daxenbichler, M. E., Spencer, G. F., Kleiman, R., VanEtten, C. H., and Wolff, I. A. 1970. Gas-liquid chromatographic determination of products from the progoitrins in crambe and rapeseed meals. Anal. Biochem. 38(2), pp. 374-382.

Lambert, J. L.,¹ Clanton, D. C.,¹ Wolff, I. A., and Mustakas, G. C. (¹University of Nebraska North Platte Station, North Platte, Nebraska). 1970. Crambe meal protein and hulls in beef cattle rations. J. Anim. Sci. 31(3), pp. 601-607.

Tookey, H. L., and Wolff, I. A. 1970. Effect of organic reducing agents and ferrous ion on thioglucosidase activity of Crambe abyssinica seed. Can. J. Biochem. 48(9), pp. 1024-1028.

NEW CROPS UTILIZATION

Problems and Objectives

Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. To develop a new crop, three basic steps are involved: (1) survey of wild plants to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical studies on components of interest to obtain clues to likely end uses; and (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential. Close cooperation is needed with plant scientists who provide assistance and advice in acquisition and selection of samples for screening and who have responsibility for subsequent agronomic research such as establishing proper cultural practices and identifying the best strains and varieties.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Identify new plant sources for industrial vegetable oils from among samples collected worldwide.
2. Determine utilization potential of oils and feed meals from selected new oilseeds.
3. Produce marketable rotenoid products from the domestic legume Tephrosia vogelii.
4. Produce papermaking pulp from kenaf.

Progress Report

A. CROPS WITH IMPROVED CONSUMER ACCEPTABILITY

1. New Sources of Industrial Vegetable Oils

Purpose: In the last 13 years at the Northern Division, some 9,300 seed samples from about 5,700 plant species have been chemically investigated, with emphasis on the nature of the seed oils. As a result of this research, numerous species are under agronomic evaluation as potential new crops; one new oilseed (crambe) has entered commercial production, and basic findings of high significance in natural products chemistry have been made. Fifty-five fatty acids not known to occur in seed oils, or not known to occur at all in nature, have been discovered, isolated, and fully identified. Species

have been found that produce seed oils like those of (imported) coconut, palm kernel, castor, tung, and rapeseed. Also, additional species were found that had seed oils like those of flaxseed and safflower, some of which may prove more widely adapted or more productive than those present crops. Many of the seeds and oil-free meals were found rich in protein; a number contained water-soluble gums of value as paper additives. Examination of 5,700 species represents a significant effort but is only a beginning toward the opportunity of finding valuable chemicals among the 250,000 known species of higher plants. The continuing high rate of discovery of lipids having new and unusual structures among previously uninvestigated species offers promise of equally rewarding results from future investigations.

Progress: Of 29 species of *Trigonella*, and 12 from related genera, none had seed richer in diosgenin than the 0.5-0.8% in those from *T. foenum-graecum*. Formation of epoxy acids was shown to occur in stored seed of *Anemone coronaria*. *Linum mucronatum* oil contains 15% of ricinoleic acid. The number of species screened for L-dopa was increased to 724 in 135 plant families, but no significant amount was found outside the genus *Mucuna*. An improved procedure for isolation of L-dopa was developed (see pages 16 and 18 for more details of recent achievements).

Evaluation of crossed breeding lines of *Brassica* continues at Oregon State University, and tests of additional accessions for adaptation and seed production are underway. Glucosinolate analyses have been made on 191 samples, mostly potential breeding material but including crosses of seven cultivars with the low-glucosinolate *B. napus* variety, Bronowski.

2. Tumor-Inhibiting Substances

Purpose: Present methods of treatment of cancer include surgery, exposure to radiation, and chemotherapy. Of these treatments, the chemotherapeutic approach is the least developed; yet it affords the greatest ultimate hope for some cases of internal cancer, including leukemia, which are essentially hopeless at present.

Thousands of samples of plant seeds representing an unusually diverse botanical spectrum are available at the Northern Division. These seeds were acquired for a chemical screening program directed towards new industrial crops. The very limited testing that has so far been possible with extracts of these seeds has revealed "confirmed actives" in tests conducted under the direction of the Cancer Chemotherapy National Service Center, National Cancer Institute, NIH.

One of the "confirmed actives" was an extract of the seed of a yew-like evergreen shrub, Cephalotaxus harringtonia. The active principle, which also occurs in other parts of the plant, is an alkaloid that has been isolated and named "harringtonine." This new compound slows the progress of experimental leukemia in laboratory mice. It is effective at very low dosages, well below the level of toxicity to the animals. Under preferred conditions of administering the drug, treated leukemic mice lived more than twice as long as untreated mice. Harringtonine is undergoing more detailed chemical and pharmacological study.

Progress: One thousand pounds of Cephalotaxus harringtonia fastigiata was processed in the pilot plant to obtain sufficient amounts of the alkaloids harringtonine, homoharringtonine, and isoharringtonine for National Cancer Institute, to begin clinical testing. A procedure was developed for the extraction and isolation of Cephalotaxus alkaloids.

Several new homoerythrina alkaloids have been isolated from Cephalotaxus extracts. These co-occur with the active antitumor alkaloids. A pilot-plant isolation has yielded gram quantities of three active alkaloids. Potamogeton ferrugineus seed oil contains 21% of a previously unknown diterpene. The major cyanolipid (58%) found in Schleichera trijuga (Kusum) seed oil is the same as that isolated previously from Cordia verbenacea and other seed oils. The erucoyl groups of Limnanthes douglasii seed were found predominantly in the 2-position. Seed extracts from one species of Indigofera contained the toxin indospicine and from two others an unknown amino acid. Of two new amino acids isolated from seed of Staphylea pinnata, one has been characterized as an N(5)-substituted derivative of gamma-hydroxyglutamine.

3. Rotenoids from Tephrosia vogelii

Purpose: As an insecticidal and piscicidal agent, rotenone has the widely recognized advantages of lack of persistence, failure to induce biological resistance, and reputation for low toxicity to mammals. For these reasons, rotenone-containing formulations enjoy a degree of consumer preference for home use, in gardens, and for clearing lakes and ponds of rough fish before restocking. The quantity of rotenoids sold each year in the U.S. varies markedly because the cost of rotenoids, which are obtained from foreign sources, is high and unstable in comparison to domestic synthetic competitive products and because of fluctuations and uncertainties in supply. A satisfactory domestic source of rotenoids would eliminate these disadvantages and promote the use of rotenoids in preference to synthetics that present health and pollution hazards.

Progress: In pilot-scale studies, a commercial specific gravity separator, which operates on the air-flotation principle, fractionated fresh T. vogelii material as efficiently as dry material. A chop length of 1 inch allowed satisfactory separations of leaflets from stem-petioles without undue contamination by petioles attached to leaflets. A shorter chop had little or no effect on the separation. The purification of deguelin for biological testing is complicated by photochemical transformations of the rotenoid. Degradation products include tephrosin and dehydrodeguelin, as expected, but not in sufficient quantities to account for deguelin losses. In the dark, deguelin stability is affected by solvent composition and decomposition occurs in acetone but not in benzene or chloroform. Polar contaminants can be separated from deguelin by chromatography on silica gel, and traces of rotenone are removable through formation of a mercuric ion adduct. Although deguelin does not readily form a mercury adduct, treatment with mercuric ions apparently destabilizes it and causes an unidentified impurity to appear. A method being developed allows direct analysis of underivatized rotenoids by gas-liquid chromatography.

At the University of Bombay, Bombay, India, under a PL 480 grant, work has been initiated on Artemisia vulgaris Linn., Origanum marjorana Linn., Pogastanum heyneanus, Acorus calamus, Gloriosa superba Linn., and Nigella sativa. Steam distillates and polar and nonpolar solvent extracts of various parts of these plants have been made for biological testing and chemical examination. For the biological tests small colonies have been started of several species of stored grain insects, namely, Trigoderma granaria, Corcyra cephalonica, Rhizopetha dominica, Brucis sinasis, and Tribolium castaneum. Assay procedures for insecticidal, repellent, and attractant activities are being set up with these insects.

4. Kenaf for Paper

Purpose: Kenaf is an annual pulp crop that shows great promise as a supplement to, or replacement for, Southern hardwood pulpwood. However, despite research conducted to date, information gaps exist in many aspects of its production and utilization. Consequently, it is not yet possible to specify a completely satisfactory and practical sequence of operations for proceeding from kenaf in the field through its mill conversion to fully acceptable commercial-grade papers. Some of the areas requiring further study include removal of undesirable constituents, continuous pulping, technology for pulping green, field-dried or preserved kenaf, bleaching, and refining.

Progress: Consideration of material losses before pulping and of chemical requirements for the pulping step, as well as pulp yields and strength properties, makes it appear advantageous to use a protective cover during simulated mill-type storage of baled field-dried kenaf. An extension of this earlier storage study has been initiated to include rigid control of material balances, effects of different

covering materials, and influence of initial kenaf moisture content. Some entries have been found with average fiber lengths of up to 0.78 mm. contrasted to the more common 0.6 mm. Preliminary data reveal significant differences in the responses of separated woody core and bast fractions to processing chemicals and mechanical treatments and in the chemical composition of the two fractions.

Pulping of kenaf in a 65-cubic-foot digester has been studied under a contract with the Herty Foundation, Savannah, Georgia. Treatments evaluated included two-stage cooking with neutral sulfite, kraft, soda, and magnesium bisulfite chemicals, and one-stage cooking with soda. Yields of crude pulps from the two-stage treatments were: Neutral sulfite, 49.3%; soda, 43.3%; and magnesium bisulfite, 44.5%. Yield from the kraft cooking was not reported; that from the single-stage soda treatment was about 2% greater than from the two-stage technique. Response of these pulps to bleaching varied with the cooking treatment. Although yields of crude pulps for the neutral-sulfite and magnesium bisulfite treatments differed by about 5%, the yields of bleached pulps were the same (42.3%) with minor differences in brightness. Strength indices for the neutral sulfite pulp were significantly greater than those for the magnesium bisulfite pulp for which the indices were also greatly less than for the kraft and soda pulps. Although the indicated demand for and actual consumption of chlorine was least for the kraft pulp, the Photovolt brightness attained for this pulp was least (82%); brightness for the other pulps differed slightly (90-92%).

Publications and Patents

A. CROPS WITH IMPROVED CONSUMER ACCEPTABILITY

1. New Sources of Industrial Vegetable Oils

Clark, T. F., Cunningham, R. L., and Wolff, I. A. 1971. A search for new fiber crops. XIV. Bond paper containing continuously pulped kenaf. Tappi 54(1), pp. 63-65.

Cunningham, R. L., Clark, T. F., Kwolek, W. F.,¹ Wolff, I. A., and Jones, Q.² (¹USDA Biometrical Services, Peoria, Illinois; ²USDA Crops Research Division, Beltsville, Maryland). 1970. A search for new fiber crops. XIII. Laboratory-scale pulping studies continued. Tappi 53(9), pp. 1697-1700.

Earle, F. R. 1970. Epoxy oils from plant seeds. J. Amer. Oil Chem. Soc. 47(12), pp. 510-513.

- Mikolajczak, K. L., and Smith, C. R., Jr. 1971. Cyanolipids of Kusum (Schleichera trijuga) seed oil. *Lipids* 6(5), pp. 349-350.
- Mikolajczak, K. L., Smith, C. R., Jr., and Tjarks, L. W. 1970. Cyanolipids of Stocksia brahuica Benth. seed oil. *Biochim. Biophys. Acta* 210(2), pp. 305-314.
- Mikolajczak, K. L., Smith, C. R., Jr., and Tjarks, L. W. 1970. Cyanolipids of Koelreuteria paniculata Laxm. seed oil. *Lipids* 5(8), pp. 672-677.
- Mikolajczak, K. L., Smith, C. R., Jr., and Tjarks, L. W. 1970. Cyanolipids of Cardiospermum halicacabum L. and other sapindaceous seed oils. *Lipids* 5(10), pp. 812-817.
- Miwa, T. K. 1971. Jojoba oil wax esters and derived fatty acids and alcohols: Gas chromatographic analyses. *J. Amer. Oil Chem. Soc.* 48(6), pp. 259-264.
- Phillips, B. E., and Smith, C. R., Jr. 1970. Glycerides of Monnina emarginata seed oil. *Biochim. Biophys. Acta* 218(1), pp. 71-82.
- Phillips, B. E., Smith, C. R., Jr., and Tallent, W. H. 1971. Glycerides of Limnanthes douglasii seed oil. *Lipids* 6(2), pp. 93-99.
- Phillips, B. E., Smith, C. R., Jr., and Tjarks, L. W. 1970. Fatty acids of Monnina emarginata seed oil. *Biochim. Biophys. Acta* 210(3), pp. 353-359.
- Smith, C. R., Jr. 1970. Occurrence of unusual fatty acids in plants. *Progr. Chem. Fats and Other Lipids* 11 (Pt. 1), pp. 137-177.
- White, G. A.,¹ and Earle, F. R. (¹USDA Crops Research Division, Beltsville, Maryland). 1971. Vernonia anthelmintica: A potential seed oil source of epoxy acid. IV. Effects of line, harvest date, and seed storage on quantity and quality of oil. *Agron. J.* 63, pp. 441-443.
- White, G. A.,¹ Willingham, B. C.,² Skrdla, W. H.,³ Massey, J. H.,⁴ Higgins, J. J.,⁵ Calhoun, W.,⁶ Davis, A. M.,⁷ Dolan, D. D.,⁸ and Earle, F. R. (¹USDA Crops Research Division, Beltsville, Maryland; ²USDA Crops Research Division, Chico, California; ³USDA Crops Research Division, Ames, Iowa; ⁴Georgia Agricultural Experiment Station, Experiment, Georgia; ⁵USDA Crops Research Division, Glenn Dale, Maryland; ⁶Oregon State University, Corvallis, Oregon; (continued to next page)

⁷USDA Crops Research Division, Pullman, Washington; ⁸USDA Crops Research Division, Geneva, New York). 1971. Agronomic evaluation of prospective new crop species. Econ. Bot. 25(1), pp. 22-43.

Wolff, I. A., and Kwolek, W. F.¹ (¹USDA Biometrical Services, Peoria, Illinois). 1971. Lipids of the Leguminosae. In "Chemotaxonomy of the Leguminosae," eds. J. B. Harborne, D. Boulter, and B. L. Turner, chap. 6, pp. 231-255. London.

2. Tumor-Inhibiting Substances

None.

3. Rotenoids from Tephrosia vogelii

Delfel, N. E. Aug. 4, 1970. Device for applying a coating of varying thickness. U.S. Patent 3,522,792.

Delfel, N. E. June 15, 1971. Dual thickness TLC plates and device therefor. U.S. Patent 3,585,129.

4. Kenaf for Paper

Clark, T. F., and Bagby, M. O. 1970. Kenaf and other nonwood species for papermaking. Indian Pulp and Paper Technical Association, Vol. VII (Conf. Suppl.), pp. 16-26.

Clark, T. F., Cunningham, R. L., Lindenfelser, L. A., Wolff, I. A.,¹ and Cummins, D. G.² (¹Eastern Marketing and Nutrition Research Division, Philadelphia, Pennsylvania; ²University of Georgia Agricultural Experiment Station, Experiment, Georgia). 1970. A search for new fiber crops. XVI. Kenaf storage. In "Non-Wood Fiber Pulping--A Progress Report," CA (Committee Assignment) Report No. 34, Technical Association of the Pulp and Paper Industry, New York, pp. 107-132.

5. Improving Protein Feed Supplements

Appelqvist, L.-A. (Swedish Seed Association, Svalof, Sweden). 1970. Lipids in Cruciferae VI: The fatty acid composition of seeds of some cultivated Brassica species and of Sinapis alba L. Fette, Seifen, Anstrichm. 72(9), pp. 783-792.*

*Research supported by PL 480 funds.

FORAGE UTILIZATION (NORTHERN REGION)

Problems and Objectives

Tall fescue grass is grown extensively in the Southeast, in the Inter-mountain States, and in the Pacific Northwest as a forage crop for cattle and other domestic animals. It has excellent agronomic characteristics, producing well on marginal land and remaining green during cool weather when other grasses are dormant. Thirty-five to fifty million acres of fescue are grown for forage use in the southeastern part of the United States alone. Cattle grazing on pasture that is predominately tall fescue sometimes develop a disease known as "fescue foot." In severe attacks, animals become emaciated and frequently die. Elimination of this disease would prevent an estimated average economic loss of about \$5 million annually.

The major objectives of current research are to discover the cause of fescue foot disease and to develop and evaluate alternate ways for its prevention.

Progress Report

A. NEW AND IMPROVED FEED PRODUCTS

1. Mycotoxins Associated With Tall Fescue

Purpose: Initial studies on the cause of toxicity of tall fescue emphasized the preparation and fractionation of various extracts of tall fescue plants from toxic fields. However, the sporadic nature of fescue foot outbreaks then suggested involvement of fungi in the etiology of the disease. Extracts of cultures of several fungi isolated from samples of fescue from toxic fields were found to produce toxic responses in rabbits and mice. In current research, fungi found on toxic fescue are being studied to determine those producing mycotoxins. These mycotoxins are concentrated or isolated and their effects on test animals are determined.

Progress: Intra-ruminal administration of 4-acetamido-4-hydroxy-2-butenic acid γ -lactone (butenolide) to cattle at daily rate of 1 g./100 lb. body weight resulted in signs of tail necrosis and eventual death of cattle maintained on timothy hay. Complete pathology reports on these animals are in preparation. Other treatments included in the experiment were butenolide administration to animals on tall fescue hay and administration of a killed Fusarium tricinatum culture to cattle maintained on timothy or on tall fescue hays. Data are still incomplete on these phases of the experiment, which is to be terminated in the near future.

A third toxin, 15-acetoxy-8- α -(3-methylbutyryloxy)-12,13-epoxytrichothec-9-en-3- α -4- β -diol (HT-2 toxin) was isolated from F. tricinctum cultures. Two hydrolysis products of 4-acetamido-4-hydroxy-2-butenic acid γ -lactone (butenolide), cis- and trans- β -formylacrylic acids, were prepared in reasonable yields by photo-oxidation of furfural. Butenolide per se is apparently not produced by F. tricinctum growing on tall fescue, and production of the toxin by the organism growing on Sabouraud's agar is prevented by the presence of fescue hay infusion. Suitable quantities of butenolide and of F. tricinctum cultured on fescue hay were prepared for cattle feeding tests.

In an examination of 37 strains of Fusarium for fungistatic and phytotoxic properties, culture extracts of 20 strains inhibited growth of Rhodotorula rubra and those of 21 strains inhibited pea seed germination. A very high correlation was demonstrated between TLC detection of T-2 toxin and inhibition of R. rubra growth by culture extracts. One strain seems to produce a toxin other than T-2 or butenolide. Eighteen of 136 Fusaria examined were not only fungistatic to R. rubra and Penicillium digitatum but also bacteriostatic to Bacillus subtilis and Cellulomonas biozotea. All 18 strains produced T-2 and an unidentified antibacterial substance.

Publications

A. NEW AND IMPROVED FEED PRODUCTS

1. Mycotoxins Associated With Tall Fescue

Burmeister, H. R. 1971. T-2 toxin production by Fusarium tricinctum on solid substrate. Appl. Microbiol. 21(4), pp. 739-742.

Burmeister, H. R., Ellis, J. J., and Yates, S. G. 1971. Correlation of biological to chromatographic data for two mycotoxins elaborated by Fusarium. Appl. Microbiol. 21(4), pp. 673-675.

Burmeister, H. R., and Hesseltine, C. W. 1970. Biological assays for two mycotoxins produced by Fusarium tricinctum. Appl. Microbiol. 20(3), pp. 437-440.

Ellis, J. J., and Yates, S. G. 1971. Mycotoxins of fungi from fescue. Econ. Bot. 25(1), pp. 1-5.

Grove, M. D., Yates, S. G., Tallent, W. H., Ellis, J. J., Wolff, I. A., Kosuri, N. R.,¹ and Nichols, R. E.¹ (¹University of Wisconsin, Madison, Wisconsin). 1970. Mycotoxins produced by Fusarium tricinctum as possible causes of cattle disease. J. Agr. Food Chem. 18(4), pp. 734-736.

Kosuri, N. R.,¹ Grove, M. D., Yates, S. G., Tallent, W. H., Ellis, J. J., Wolff, I. A., and Nichols, R. E.¹ (¹University of Wisconsin, Madison, Wisconsin). 1970. Response of cattle to mycotoxins of Fusarium tricinctum isolated from corn and fescue. J. Amer. Vet. Med. Ass. 157(7), pp. 938-940.

